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STUDIES ON THE DNA CONTENT, DRY MASS AND OPTICAL AREA OF MORPHOLOGICALLY NORMAL AND ABNORMAL BULL SPERMATOZOAL HEADS*)

By

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In ejaculated semen from highly fertile bulls, abnormal head forms constitute five to 20 per cent of the total number of spermatozoal heads, while even higher percentages are commonly found in ejaculates from bulls with certain types of reduced fertility. When performing a cytophotometric study on a population of spermatozoal heads, it is necessary to consider the question of whether or not differences exist between morphologically abnormal heads and their normal counterparts within the cytophotometric parameter to be investigated. This publication is the result of quantitative cytophotometric studies which were performed on ejaculated bull spermatozoal heads in order to determine if differences do exist between morphologically normal and abnormal forms with respect to DNA content, total dry mass and optical area.

MATERIALS AND METHODS

Two young bulls of the Swedish Red and White breed, admitted for study to the stationary clinic of the Obstetrics and

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Gynecology Department, Royal Veterinary College, Stockholm because of poor semen quality, were used for this investigation. Both bulls were judged to be free from all infectious diseases known to cause infertility. Ten months before admission, bull I had served six heifers all of which returned to estrus. One month before admission, this animal was examined by a veterinarian and carbolfuchsin-eosin stained semen smears revealed excessive numbers (c. 40 %) of morphologically abnormal spermatozoal head forms. Bull II was examined by a veterinarian at three months and one month prior to admission to the clinic. Morphological examination of stained semen smears demonstrated on both occasions about 30 per cent abnormal head forms.

Spermatozoa from two ejaculates from each bull, collected on separate days by using an artificial vagina, were used for cytophotometry. Each ejaculate was evaluated for total volume, per cent initial motility of spermatozoa and per cent abnormal head forms as described by *Lagerlöf* (1934, 1964). The concentration of spermatozoa was determined by the use of a hemocytometer (*Bane* 1952). The per cent live spermatozoa was estimated soon after ejaculation using the supravital staining technique of *Hancock* (1957) and counting a total of 400 cells. This same technique and number of cells were used just prior to fixation on a small sample of the washed spermatozoa (see below) in order to determine the per cent live cells in each of the subpopulations prepared for cytophotometry.

Immediately after collection, a small (0.2 ml) amount of semen was pipetted into 0.4 ml of a buffered balanced salt solution (*Mann* 1964) and thoroughly mixed by gentle agitation. The suspension was then centrifuged for four minutes at a slow speed (c. 40 $\times$ *g*). The supernatant fluid was withdrawn with a thin drawn glass pipette and discarded. Addition of 0.4 ml of the salt solution, resuspension, recentrifugation and withdrawal of the supernatant fluid was then done as the second washing. After a third washing, the remaining sediment of recentrifuged cells was suspended in one ml of neutral buffered formaldehyde solution (*Lillie* 1948). After a fixation time of ten minutes and recentrifugation, a portion of the spermatozoa found at the bottom of the centrifuge tube was transferred with a pipette to a clean Bürker haemocytometer cover slide or a fused quartz slide. This drop of spermatozoal suspension was then smeared and allowed to air dry one minute. Preparations made in this manner,

with very few exceptions, had suitable numbers of undistorted spermatozoa lying free on their broad flat surfaces, evenly distributed over a clear background. Duplicate preparations were always made for each of the cytophotometric parameters investigated.

Lagerlöf's (1934, 1964) system for the morphological classification of carbolfuchsin-eosin stained spermatozoa was the basis of the morphological grouping used during cytophotometry. The classification of morphologically abnormal as used in this investigation is composed of three types of head forms. These types were chosen because they formed a large portion of each

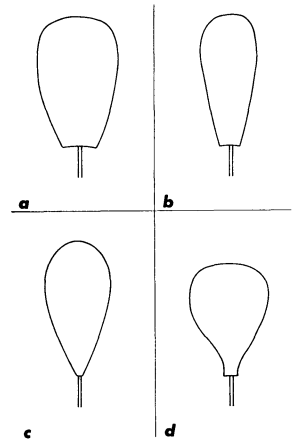

Figure 1. A schematic drawing illustrating morphologically normal (a) and abnormal (b) (c) (d) bull spermatozoal heads.

ejaculate's abnormal head forms (carbolfuchsin-eosin stained smears) (Table 1). Although the three types of abnormal heads measured (Fig. 1b, c, d) were similar in general appearance to the narrow, narrow at the base and pear shaped heads of *Lagerlöf*'s classification, they were not necessarily identical since different stains and optical systems than those used by *Lagerlöf* were utilized for cell identification during cytophotometry. Approximately equal numbers of morphologically normal (Fig. 1a) and morphologically abnormal spermatozoal heads were measured. In general, 25 heads of each of the two classifications were measured on each of the duplicated preparations. Within the classification of abnormal, approximately equal numbers of the three types were measured.

Smears to be analyzed in monochromatic ultraviolet light (u. v.) at 2650Å and 2800Å were made on fused quartz slides and placed in an excess of redistilled glycerol (Merck: n = 1.454) for two hours. After allowing each slide to drain briefly, a thin round quartz coverslip was placed over the film of glycerol that remained on the slide and was secured by melted paraffin. All the measurements of u. v. absorbing substances in 380 spermatozoal heads were done on a rapid high-resolution scanning and integrating cytophotometer (*Lomakka* 1965a).

Smears intended for Feulgen microspectrophotometry were made on haemacytometer cover slides and stained at room temperature for 60 minutes with a fuchsin solution prepared according to *Coleman* (1938). Prior to immersion in the staining reagent, acid hydrolysis had been effected in N-HCl at 60°C for nine minutes. Gurr's DePeX ®(n = 1.528) was used as the mounting medium. Two slides upon which fresh human peripheral blood had been smeared and fixed for ten minutes in neutral buffered formaldehyde solution (*Lillie*) were included as a stain reference system each time a set of semen smears was processed. Absorption of light at the 5460Å wave length by 470 Feulgen stained spermatozoal nuclei and 150 similarly stained polymorphonuclear leukocytes was measured on the rapid high-resolution scanning and integrating ultramicrospectrophotometer described by *Caspersson & Lomakka* (1962). All mean light absorption values for the Feulgen stained spermatozoal nuclei herein reported have been adjusted through the use of the polymorphonuclear leukocyte stain reference system.

Data obtained by the technique for the measurement of light absorption (i. e., microspectrophotometry) are reported as total extinction at the wave lengths employed and are expressed in square micra. Total extinction is defined as the surface integral of the optical density. In this publication, no meaningful correlation exists between the units used to express the results of the u. v. measurements and the Feulgen measurements. All total extinction expressions are uncorrected for non-specific light losses since these losses, as estimated by total extinction at 3150Å where true absorption by cellular material is negligible (*Caspersson* 1940), rarely exceeded five per cent and generally were closer to one or two per cent of the uncorrected 2650Å values.

Preparations of spermatozoa on haemacytometer cover slides intended for microinterferometric determinations were placed

into sterile glass distilled water (n = 1.333). They were aged for two hours and then glass coverslips were placed on top of the thin layer of water which remained on the slides and fastened with melted paraffin. The surface integrals of the optical path difference between the heads and their backgrounds, values which are proportional to the total dry mass (*Carlson & Gledhill* 1966) of the 393 heads, were measured in a rapid scanning and integrating microinterferometer (*Lomakka* 1965b). The microinterferometrically obtained data are expressed in units of 10^{-12}cm^3.

The preparations used for microinterferometry were also used to determine the optical area in square micra of 239 spermatozoal heads. The instrument used to obtain these measurements was the recording microplanimeter described by *Caspersson et al.* (1960).

RESULTS

The volume of each ejaculate and its respective concentration of spermatozoa were normal (Table 1). There was a reduced frequency of motile spermatozoa in the number 1 ejaculate from bull I while the other ejaculates were within the generally accepted limits of normal physiological variation. The per cent live spermatozoa was normal in all ejaculates. After the washing procedure, a slight drop in per cent live cells was noted but even these slightly reduced frequencies are acceptable as normal. Carbolfuchsin-eosin stained smears of the number 1 ejaculate from bull I demonstrated a high per cent of morphologically abnormal head forms; however, the number 2 ejaculate did not contain excessive numbers of these forms. The per cent of morphologi-

Table 1. Summary of semen characteristics.

Bull	Ejaculate number	Volume ml	Sperm conc. 10^6/mm^3	Motility %	Live sperm *) %	Total abnormal head forms %	Narrow, narrow at the base and pear shaped %
I	1	3.0	0.76	30	87 (83)	31	19
	2	5.6	0.96	60	88 (78)	15	12
II	1	4.4	0.83	70	91 (85)	26	23
	2	3.7	0.81	60	87 (74)	29	24

*) The per cent of live spermatozoa after washing and immediately before fixation is given within parentheses

Table 2. Summary of cytophotometric results for individual ejaculates.

	Bull I							
	Ejaculate 1				Ejaculate 2			
	Normal		Abnormal		Normal		Abnormal	
Parameter	Mean[a]	c.v.[b]	Mean	c.v.	Mean	c.v.	Mean	c.v.
Total ext. at 2650Å (μ^2)	6.65	*2.11*	6.69	*1.49*	6.66	*3.75*	6.62	*3.32*
Total ext. at 2800Å (μ^2)	4.75	*2.95*	4.75	*2.95*	4.79	*4.59*	4.76	*4.62*
Total ext. at 5460Å (μ^2)	6.19	*4.04*	5.99	*3.67*	4.95	*4.04*	4.70	*5.96*
Surface integr. o.p.d. (10^{-12}cm^3)	1.51	*4.64*	1.52	*4.61*	1.60	*6.25*	1.50	*6.67*
Optical area (μ^2)	42.5	*4.00*	37.0	*10.73*	43.3	*4.02*	39.8	*7.66*

	Bull II							
	Ejaculate 1				Ejaculate 2			
	Normal		Abnormal		Normal		Abnormal	
Parameter	Mean	c.v.	Mean	c.v.	Mean	c.v.	Mean	c.v.
Total ext. at 2650Å (μ^2)	6.57	*3.04*	6.53	*3.37*	6.73	*3.27*	6.70	*4.18*
Total ext. at 2800Å (μ^2)	4.69	*3.62*	4.71	*4.67*	4.85	*4.54*	4.84	*5.17*
Total ext. at 5460Å (μ^2)	4.85	*3.51*	4.67	*4.71*	3.67	*20.89*	3.33	*20.42*
Surface integr. o.p.d. (10^{-12}cm^3)	1.55	*5.81*	1.46	*6.85*	1.51	*5.30*	1.45	*5.52*
Optical area (μ^2)	42.8	*5.02*	39.6	*8.96*	40.0	*6.23*	38.3	*7.83*

a) Mean per head
b) Coefficient of variation

cally abnormal head forms was high in both of the ejaculates from bull II. In all ejaculates, the three types of abnormal head forms chosen for cytophotometric study were responsible for a large portion of the total per cent of heads with abnormal morphology.

In order that an idea of the relative similarity between ejaculates may be had, a list of their cytophotometric mean values and coefficients of variation is presented in Table 2. For each set of the cytophotometric results presented in Table 3, all the measurements on morphologically normal forms have been grouped together as have all the measurements on the three

Table 3. Summary of morphologically grouped cytophotometric results.

Parameter	Morphologically normal: No. of heads	Mean per head ± Standard error
Total ext. at 2650Å	200	6.65 ± 0.01 μ^2
Total ext. at 2800Å	200	4.77 ± 0.01 μ^2
Total ext. at 5460Å	235	4.94 ± 0.06 μ^2
Surface integr. o. p. d.	200	1.54 ± 0.007×10^{-12}cm³
Optical area	119	42.2 ± 0.2 μ^2
Parameter	**Morphologically abnormal: No. of heads**	**Mean per head ± Standard error**
Total ext. at 2650Å	180	6.62 ± 0.02 μ^2
Total ext. at 2800Å	180	4.76 ± 0.02 μ^2
Total ext. at 5460Å	235	4.70 ± 0.07 μ^2
Surface integr. o. p. d.	193	1.48 ± 0.007×10^{-12}cm³
Optical area	120	38.7 ± 0.3 μ^2

morphologically abnormal head forms. This has been done without regard to the ejaculate or bull of origin.

Neither the 2650Å nor the 2800Å mean total extinction value for morphologically normal spermatozoal heads was significantly different from the corresponding mean value for abnormal heads when tested by an analysis-of-variance (Table 4) (*Bonnier & Tedin* 1940). A significant difference ($0.01 > P > 0.001$) was found to exist between the respective mean values for 5460Å total extinction of morphologically normal and abnormal Feulgen stained nuclei. A highly significant difference ($P < 0.001$) was found between the mean values of the two morphological classifications with respect to both the surface integrals of the optical path difference and the optical area.

Table 4. Summary of statistical tests concerning the means of the two morphological classifications.

	df[a]	Sum of squares	Mean square	Quotient	
Analysis-of-variance					
2650Å					
Between classif.	1	0.06	0.06	1.2	
Within „	378	18.01	0.05		
2800Å					
Between classif.	1	0.01	0.01	0.025	
Within „	378	15.66	0.04		
5460Å					
Between classif.	1	6.84	6.84	6.90**	
Within „	468	462.20	0.99		
Surface integr. o.p.d.					
Between classif.	1	0.31	0.31	34.44***	
Within „	391	3.70	0.009		
Analysis of means with unequal variances					
Optical area	Mean	Difference	Number of heads	Variance	
Morph. normal	42.2	3.50	119	5.73	t = 8.95***
„ abnormal	38.7		120	12.66	

[a] degrees of freedom
** $0.01 > P > 0.001$
*** $P < 0.001$

To discover if the variances of each morphologically classified group of heads differed within each of the cytophotometric parameters, a test of the equality of the variances was performed (*Dixon & Massey* 1957). The only parameter in which there was a significant difference between the variances was that of optical area (Table 5). This difference necessitated the use of an alternate method for testing the means of the two morphological classifications in this parameter (*Dixon & Massey*) (Table 4).

DISCUSSION

The fundamental evidence which demonstrated that quantitative estimations of the total nucleic acid content and the content of certain of the cyclic amino acid containing proteins in a single cell or cell part could be made by microspectrophotometry was presented by *Caspersson* in 1936. Reports of chemical

Table 5. Summary of tests of equality of the variances in morphological classifications.

Parameter	Morphological classification	Coefficient of variation	Variance	df [a]	F ratio
Total ext. at 2650Å	normal	3.16	0.0458	199	0.923
	abnormal	3.32	0.0496	179	
Total ext. at 2800Å	normal	4.19	0.0395	199	0.908
	abnormal	4.41	0.0435	179	
Total ext. at 5460Å	normal	19.8	0.952	234	0.933
	abnormal	21.5	1.020	234	
Surface integr. o. p. d.	normal	6.49	0.00920	199	0.945
	abnormal	6.76	0.00974	192	
Optical area	normal	5.67	5.730	118	0.453***
	abnormal	9.21	12.660	119	

[a] degrees of freedom
*** $P < 0.001$

analyses which state that spermatozoa of several species of mammals and three species of fish are remarkable for their virtual lack of RNA have been cited by *Bishop & Walton* (1960). *Bhargava* (1964) concluded that the RNA content of bull spermatozoa does not amount to more than 0.02 per cent of the DNA content. For the purposes of this discussion, it can be assumed that practically all of the absorption at 2650Å in the heads of unstained and glycerol immersed spermatozoa is due to DNA.

Since the structurally aromatic amino acids tyrosine, tryptophan and phenylalanine are characteristically present in small amounts in spermatozoal heads (*Mann* 1964, *Henricks & Mayer* 1965), it is logical to credit nearly all of the absorption of 2800Å light by spermatozoal heads to the greatly dominating amounts of DNA and not to the proteins. An increase in the amount of these cyclic amino acids would be reflected by a reduction in the $\frac{2650}{2800}$ Å total extinction ratio although the reverse, in the case of spermatozoa, would have only a slight influence. The occurrence of dissimilar ratios for the two morphological groups investigated could be of significance and certainly would be of interest. No appreciable dissimilarity in the ratios was observed and the

ratios are similar to those found in the literature for comparable material (*Sandritter & Grosser* 1964).

One of the methods currently in vogue for the demonstration and cytophotometric quantitation of DNA is the nucleal or Feulgen reaction. Since the appearance of *Feulgen*'s original studies (1914, 1924) and through later studies reported by other authors on quantitative applications (see *Pollister & Ornstein* 1959), support for the following concepts has been forthcoming: *i*) a strict specificity of the Feulgen reaction for DNA under conditions which eliminate interference by other fuchsin-staining substances,

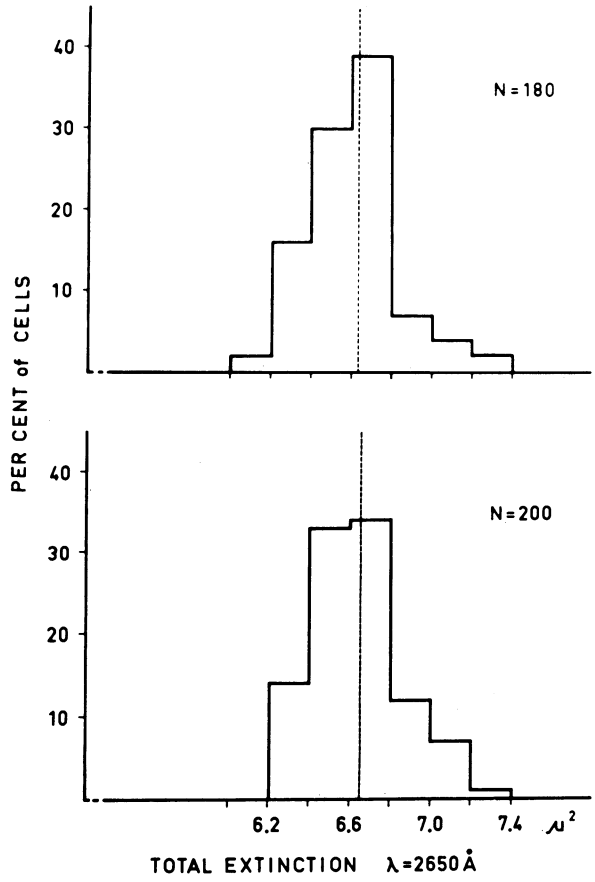

Figure 2. The frequency distributions of 2650Å total extinction values in μ^2 for morphologically normal (*lower*) and abnormal (*upper*) bull spermatozoal heads. The vertical interrupted lines represent the respective mean values and N relates the number of heads measured.

and *ii*) a proportionality between the DNA content of a cell and the amount of stain bound in it by the Feulgen reaction.

There appears to be a contradiction between the u. v. and Feulgen results reported herein. No significant difference was found to exist between the mean values for heads with normal morphology and the mean values for heads with abnormal morphology when measured at 2650Å and 2800Å. A significant difference was found, however, between the means for these two morphological classifications when the measurements were performed at 5460Å on Feulgen stained nuclei.

In a cytochemical study of bull spermiogenesis, *Gledhill et al.* (1966) have noted that 5460Å total extinction values decrease considerably during spermateliosis. This decrease is apparently not correlated to a true decrease in the amount of DNA but to

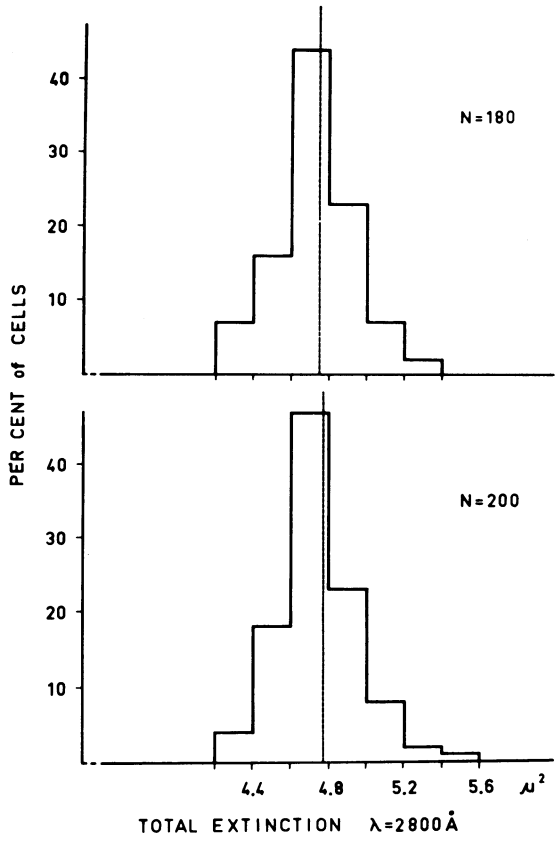

Figure 3. The frequency distributions of 2800Å total extinction values in μ^2. See the caption to Fig. 2.

qualitative changes in the basic protein bound to DNA as judged by altered dye binding properties of spermatozoal head proteins *and* to a reduction in the total number of cationic dye binding phosphate groups in the DNA. Thus, 2650Å total extinction values for cells representing various phases of spermateliosis, after treatment with RNase and cold TCA to remove RNA, remained constant.

Comparison of the frequency distributions in Figs. 2, 3 & 4 clearly shows that all of the u. v. measurements exhibit more compact distributions than do the Feulgen measurements. The coefficients of variation for 5460Å total extinction measurements, in both of the morphological classifications, are five to seven times as large as the corresponding coefficients of variation for 2650Å and 2800Å total extinction measurements (Table 5). These unusually large coefficients of variation are partially explained by the fact that the 5460Å total extinction values for both of the morphological groups varied considerably from ejaculate to ejaculate even after adjustment by use of the stain reference system (Table 2). However, even with elimination of this factor, the 5460Å measurements fluctuate more than do the u.v. measurements as is evident from the coefficients of variation for single ejaculates (Table 2).

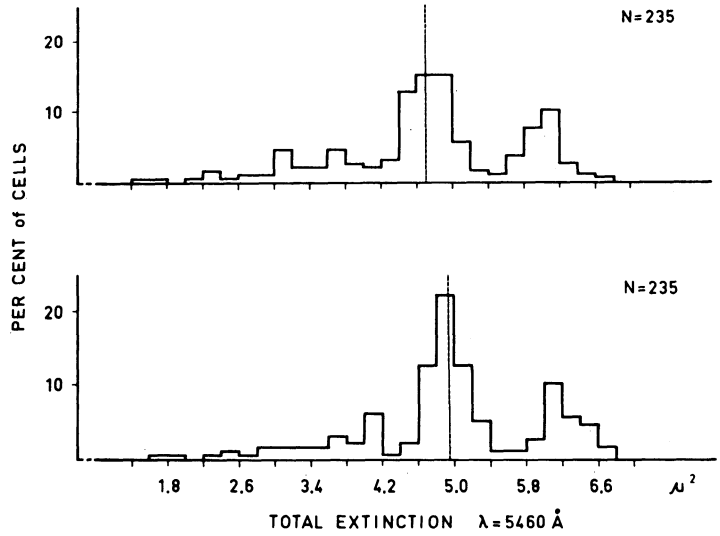

Figure 4. The frequency distributions of 5460Å total extinction values in μ^2. See the caption to Fig. 2.

It would seem, therefore, from the results at hand, that morphologically normal and abnormal heads do not contain differing mean amounts of DNA despite the observed difference in the 5460Å total extinction values. This conclusion is based on the foregoing discussion where it was noted that the mean amounts of u. v. absorbing material (practically all DNA in this case) did not differ and that the estimation of Feulgen-positive material in spermatozoal heads is apparently influenced by substances other than DNA. In addition, the smaller coefficients of variation found in the u. v. measurements seem to be relatively strong evidence in favor of the reliability of u. v. microspectrophotometry.

Microinterferometry is one of the three main methods currently being used for the determination of dry mass at the cellular or subcellular level. The other methods are soft X-ray

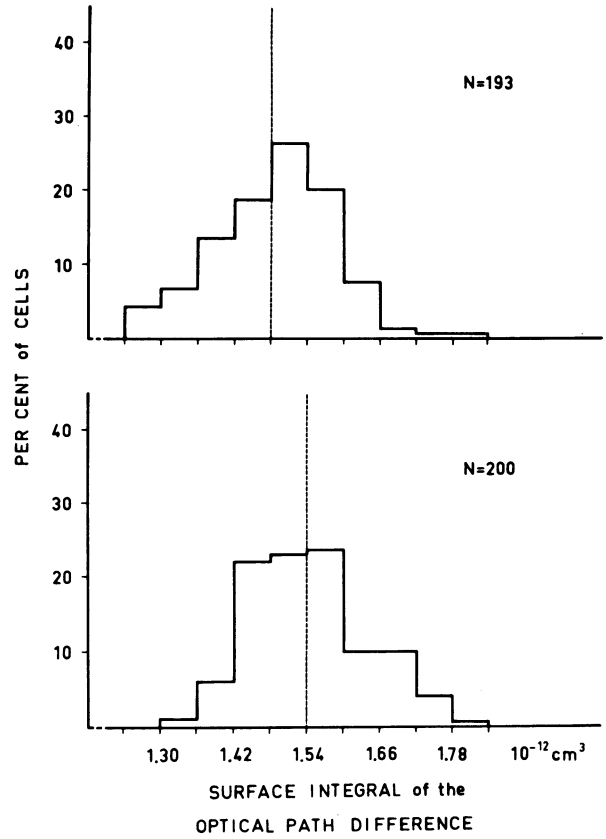

Figure 5. The frequency distributions of the surface integrals of the optical path difference $\times 10^{-12}cm^3$. See the caption to Fig. 2.

microradiography and quantitative electron microscopy. The microinterferometrically determined surface integrals of the optical path difference reported in this investigation may be converted to total dry mass in grams by dividing the surface integral of the optical path difference by 0.12 cm^3/g (*Carlson & Gledhill* 1966).

The specific factor responsible for the highly significant difference between the means of the two morphological classifications can not be identified by the available results. However, it is probable that the observed reduction in the integrated optical path difference (and therefore total dry mass) of the abnormal heads is due to an actual reduction in some type of organic material. Since the reduction is less than four per cent of the value for normal heads, it is not beyond reason to think that spermatozoal heads that are abnormally shaped might be lacking in extra-nuclear material.

During microplanimetry of the spermatozoal heads, no attempt was made to exclude the acrosome or other cytoplasmic structures. Water is known to cause swelling of the acrosome and this effect would partially account for the fact that the mean optical area for heads in both of the morphological classifications was higher than is sometimes reported in the literature. Some investigators (*Bahr & Zeitler* 1964) have estimated the area of bull spermatozoal heads excluding the acrosome while others (*Salisbury & vanDongen* 1964) have used techniques differing from those used in the present investigation.

The highly significant difference which occurred between the means of the two morphological classifications is not difficult to understand when the shapes of the abnormal head forms selected for measurement are remembered. The frequency distributions differed in appearance (Fig. 6) with a greater number of the abnormal cells having decidedly smaller optical areas. The variances in both of the morphological classifications were unequal at a highly significant probability level. There is no doubt as to the facts that the morphologically abnormal head forms measured in this study vary more and have smaller mean optical areas than do normal heads.

In this publication, the attitude has been adopted that u. v. microspectrophotometry is preferable to Feulgen microspectrophotometry for the estimation of DNA content in bull spermatozoal heads. The background to this attitude has been discussed

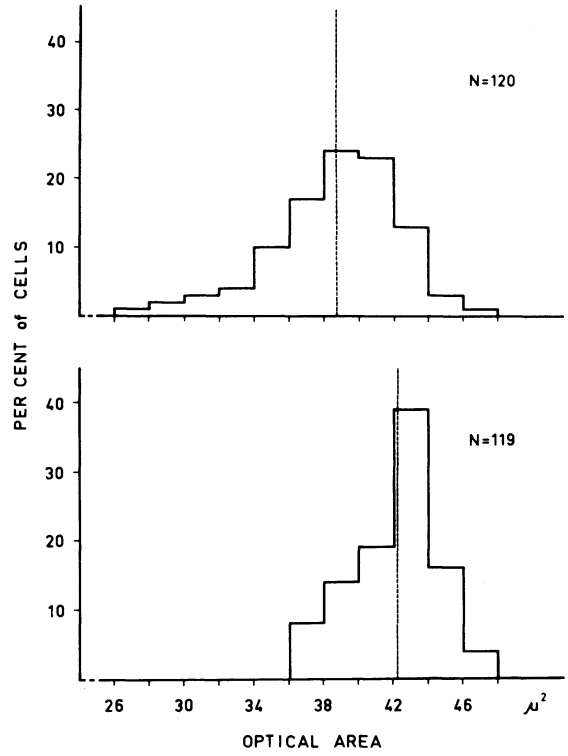

Figure 6. The frequency distributions of the optical area in μ^2. See the caption to Fig. 2.

above. The reductions in both the mean surface integral of the optical path difference and the light absorption of Feulgen stained morphologically abnormal heads are thus interpreted as being coincidental and not as reflecting a true relationship.

Based on clinical examinations and semen characteristics, bull I was suffering from mild testicular degeneration and left-sided spermiostasis. During the course of this bull's stay in the clinic, when he was on a fairly routine schedule of ejaculate collection, there was a gradual improvement in his semen quality. Bull II showed the clinical signs commonly associated with testicular degeneration. No evidence of a regenerative phase was forthcoming.

In these cytophotometric parameters, a deviation from normal levels by morphologically normal heads may be hidden by only examining spermatozoa obtained during a period of testicular degeneration since one may not safely assume that just because

a spermatozoon is morphologically normal it is normal in all respects. The comparison of such heads to morphologically normal spermatozoal heads from bulls with normal testicular function could be enlightening. The mean cytophotometric values reported in this study for the morphologically normal heads are in agreement with mean values of corresponding measurements on morphologically similar heads from fertile bulls. The results of the studies on fertile bulls will be included in a later report. Further, in bull I spermatozoal morphology normalized during the investigational period (Table 1) but this improvement in semen quality was not accompanied by a change in the u. v. total extinction values of the spermatozoal heads (Table 2).

It has been suggested by *Knudsen* (1954, 1958) that defects in the distribution of chromosomes during meiosis, resulting from a disturbance of the spindle fibers in anaphase, will give rise to the morphologically abnormal spermatozoal head forms commonly present in relatively high numbers in cases of slight testicular degeneration. *Henricson & Bäckström* (1964), in their study of the meiotic divisions in boars and bulls, reported a radical disturbance in the chromosome distribution of a totally sterile boar, possibly caused by a defective nuclear spindle mechanism. They felt that nondisjunction, and the formation of spermatozoa with faulty gene sets probably occurred. Although *Henricson & Bäckström* state that the distribution of chromosomes in anaphase can be so greatly disturbed as to result in the formation of gametes with an incorrect gene complement, all of the animals they investigated, with the exception of two boars whose spermatozoa possessed an acrosomal defect, had morphologically normal semen pictures. These authors thought it probable that the primary cause of the formation of defective gametes was to be found in non-chromosomal structures such as the meiotic division apparatus and was not due to chromosomal changes in the spermatozoal nuclei.

By accepting the DNA constancy thesis which originates from the studies of *Boivin et al.* (1948) and *Mirsky & Ris* (1949) and states that the DNA content of a nucleus is directly proportional to the number of chromosomes it contains, microspectrophotometry becomes useful in attacking the question of whether or not morphologically abnormal head forms of bull spermatozoa contain DNA amounts which correspond to varying numbers of chromosomes. Since no significant difference existed between

the variances of the measurements of u. v. absorbing substances, there is little reason to suspect that a greater frequency of imbalance of chromosomes would be found among heads with abnormal morphology than among a population of normal heads. In this context and as further support, it can be again stated that no significant differences evolved between the variances of both morphological classifications in the cytophotometric parameters of 5460Å total extinction and surface integral of the optical path difference.

CONCLUSIONS

With respect to the two morphological groups of bull spermatozoal heads which were cytophotometrically analyzed in these studies, the following conclusions are made.

1. Morphologically normal and abnormal heads do not contain differing mean amounts of DNA when measured by u.v. microspectrophotometric techniques but differences exist when DNA is estimated by Feulgen microspectrophotometry.
2. Abnormal heads have a lower mean total dry mass (surface integral of the optical path difference) than do normal heads.
3. The abnormal head forms vary more and have a smaller mean optical area than do normal heads.
4. In cytophotometric studies, errors arising from these dissimilarities can be avoided by measuring only morphologically normal spermatozoal heads.

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SUMMARY

Quantitative microspectrophotometric, microinterferometric and microplanimetric techniques were used to compare morphologically normal and abnormal bull spermatozoal heads with respect to DNA content, total dry mass and optical area. Spermatozoa were obtained from ejaculates of two young bulls with disturbances in spermatogenesis. U. v. microspectrophotometric results exhibited no difference in mean amounts of DNA between normal and abnormal spermatozoal heads. Apparently contradictory results were found with Feulgen microspectrophotometry and were discussed. Highly significant differences in mean values were observed between the normal and abnormal heads for total dry mass and optical area. The only cytophotometric parameter in which a significant difference existed between the variances of the two morphological groups was optical area.

ZUSAMMENFASSUNG

Untersuchungen über DNA-Gehalt, Trockengewicht und Fläche morphologisch normaler und anomaler Spermatozoenköpfe von Bullen.

Es wurden quantitative mikrospektrophotometrische, mikrointerferometrische und mikroplanimetrische Methoden angewandt, um morphologisch normale und anomale Spermatozoenköpfe von Bullen in Bezug auf DNA-Gehalt, Trockengewicht und Fläche zu vergleichen. Die Spermatozoen wurden von Ejakulaten zwei junger Bullen, die Störungen in der Spermatogenese aufwiesen, erhalten. U. v.-mikrospektrophotometrische Messungen ergaben keinen Unterschied in der durchschnittlichen DNA-Menge normaler und anomaler Spermatozoen-

köpfe. Dem widersprachen Resultate Feulgen-mikrospektrophotometrischer Messungen. Der Widerspruch wird diskutiert. Es wurden sehr signifikante Unterschiede in den Mittelwerten für Trockengewicht und Totalfläche zwischen normalen und anomalen Spermienköpfen gefunden. Der einzige cytophotochemische Parameter, in dem ein signifikanter Unterschied zwischen den Variationen der beiden morphologischen Gruppen zum Ausdruck kam, war die Fläche.

SAMMANFATTNING

Undersökningar över DNA-mängd, torrvikt och cellyta av morfologiskt normala och abnorma spermiehuvuden hos tjur.

Kvantitativ mikrospektrofotometri, mikrointerferometri och mikroplanimetri användes för att jämföra morfologiskt normala och abnorma spermiehuvuden hos tjur med avseende på DNA-mängd, torrvikt och cellyta. Spermierna erhölls från ejakulat av två unga tjurar med rubbning i spermatogenesen. U. v. mikrospektrofotometri visade ingen skillnad i medelvärdena för DNA-mängd mellan normala och abnorma spermiehuvuden. Till synes motsägande resultat erhölls med Feulgen mikrospektrofotometri och dessa diskuteras. Starkt signifikanta skillnader i medelvärden befanns råda mellan de normala och de abnorma spermiehuvudena med avseende på torrvikt och cellyta. Cellytan var den enda parameter i vilken de båda morfologiska grupperna visade signifikant olika varians.

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