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STUDIES ON THE DNA CONTENT, DRY MASS AND OPTICAL AREA OF EJACULATED SPERMATOZOAL HEADS FROM BULLS WITH NORMAL AND LOWERED FERTILITY*)

By

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More than a decade has passed since *Leuchtenberger et al.* (1953) first suggested that one type of human male infertility might be correlated to a deficiency of DNA in spermatozoa. This suggestion was made on the basis of data from cytophotometric analyses of Feulgen stained spermatozoa and was soon extended to include bulls (*Leuchtenberger et al.* 1956). In one part of a cytophotometric investigation on the DNA content of germ cells, *Welch & Resch* (1960) found that there was no statistically valid relationship between a small reduction in mean Feulgen-DNA content of ejaculated spermatozoa and reduced fertility in Santa Gertrudis bulls. *Parez et al.* (1960), after using quantitative Feulgen techniques, reported that spermatozoa from several bulls with poor fertility contained more DNA than did spermatozoa from bulls with normal fertility. The apparent

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Table 1. Summary of semen characteristics for individual bulls.

Bull no.	Age yrs.	No. of ejacs.	Motility %	Sperm conc. $10^6/mm^3$	Abnormal heads %	Prox. cytopl. drops. %
Lowered fertility						
1	3½	22	47	1.01	10	3
2	4½	26	32	0.97	15	3
3	4½	26	37	0.92	14	2
4	2½	25	62	1.61	9	1
5	4	30	46	1.25	11	2
6	5	30	37	0.69	16	3
7	2½	16	54	0.69	16	3
8	2½	19	38	0.68	23	4
Normal fertility						
9	5½	11	72	0.87	9	1
10	12	16	74	not regularly investig. at A.I. center		
11	10½	27	75	not regularly investig. at A.I. center		
12	8	18	71	not regularly investig. at A.I. center		
13	1½	14	61	0.52	9	3
14	2	6	68	1.70	11	1
15	15	4	65	1.42	9	1

contradiction in the findings cited above motivated the present cytophotometric study in which data on the DNA content, total dry mass and optical area of morphologically normal ejaculated spermatozoal heads were collected from bulls with lowered fertility and compared to similarly obtained data from bulls with normal fertility.

MATERIALS AND METHODS

Of the 15 Swedish Red and White breed bulls used for this study eight had been admitted because of lowered fertility to the clinic of the Obstetrics and Gynecology Department, Royal Veterinary College, Stockholm. One of the eight bulls (no. 8) had mild testicular degeneration plus left-sided spermiostasis. The reasons for the lowered fertility of the seven remaining bulls in this group of eight were unknown despite extensive clinical study including, in some cases, investigation of somatic chromosomes. With but one exception (Table 1, bull no. 4) these eight bulls were subnormal in average frequency of motile spermatozoa, but all the bulls had semen that was clinically normal in other respects according to the standards of *Lagerlöf* (1934).

T a b l e 2. Fertility rates and mean Feulgen values for individual bulls.

Bull no.	Fertility: Total breedings	Fertility: 56 day N. R. R.[a] %	Fertility: 4 week N. R. R.[a] %	Fertility: Cows pregn. rectal exam. %	Feulgen: Mean per head μ^2
Lowered fertility					
1	8[b]	—	—	13	3.98
2	63[b]	—	—	16	4.57
3	160[c]	—	—	33	3.90
4	357[c]	38	—	—	4.06
5	168[c]	—	39	—	5.24
6	214[c]	—	39	—	5.22
7	165[c]	—	—	16	4.03
8	6[b]	—	—	0	5.25
Normal fertility					
9	2814[c]	68	—	—	3.80
10	6917[c]	68	—	—	4.62
11	9451[c]	69	—	—	4.00
12	3195[c]	63	—	—	4.29
13	+	—	—	—	3.19
14	+	—	—	—	2.94
15	410[c]	—	—	57	2.20

[a] Non-return rate
[b] Natural service
[c] Artificial insemination
— not applicable
\+ Not used but approved for artificial insemination

The lowered fertility of these bulls was clearly established from breeding records, mostly from artificial insemination (A.I.) centers (Table 2). All bulls utilized were judged to be free from infectious diseases which cause infertility.

Seven bulls comprised the group termed normal. Of these, five were in regular use at A.I. centers and had average fertility rates (Table 2) which were well documented. Additionally, two young bulls which had been approved for use in A.I. in regard to semen quality and serving behavior were included in this group. Although these two bulls had not yet been used for breeding purposes, they were assumed to be normally fertile.

Ejaculated spermatozoa, following collection in an artificial vagina, were thrice washed with buffered balanced salt solution (*Mann* 1964), fixed in neutral buffered formaldehyde solution (*Lillie* 1948) and prepared for cytophotometric analyses as

described previously (*Gledhill* 1966a). The DNA content of approximately 2500 individual spermatozoal heads, as represented by total extinction at 2650Å, was determined by ultraviolet (u.v.) microspectrophotometry (*Lomakka* 1965a). With the same instrument, and immediately following 2650Å measurement of each cell, the amount of 2800Å absorbing substances (in the case of spermatozoal heads, mostly DNA) was determined. The amount of Feulgen stain in close to 2600 different spermatozoa was microspectrophotometrically estimated (*Caspersson & Lomakka* 1962) as 5460Å total extinction. The surface integral of the optical path difference (o.p.d.), a value which can be converted into total dry mass in grams by dividing the integrated o.p.d. by 0.12 cm^3/g (*Carlson & Gledhill* 1966), of nearly 2600 individual spermatozoal heads in water was microinterferometrically determined (*Lomakka* 1965b). The optical area of one broad flat aspect of slightly more than 1000 of the spermatozoal heads on the preparations used for microinterferometry was estimated by microplanimetry (*Caspersson et al.* 1960). All mean u.v. absorption measurements are uncorrected for non-specific light loss (*Caspersson* 1950) because the loss was always negligible, and all mean 5460Å total extinction values have been standardized by the use of a Feulgen stain reference system based on the amount of stain in human polymorphonuclear leukocytes (*Gledhill* 1966a). For each bull, approximately the same number of morphologically normal heads was measured in each parameter for each ejaculate and, in general, two or three ejaculates were sampled over a period of several months.

RESULTS AND DISCUSSION

When, for each of the cytophotometric parameters studied, the mean value per spermatozoal head for the eight bulls with lowered fertility (Table 2) was compared by the analysis-of-variance method (*Bonnier & Tedin* 1940) to the corresponding value for the seven normal bulls, there was only one parameter in which the difference between the means reached statistical significance. The mean 5460Å total extinction value for Feulgen stained spermatozoa from bulls with lowered fertility was significantly ($0.05 > P > 0.01$) higher than the mean value for spermatozoa from the normal bulls. Previously (*Gledhill* 1966b), higher mean 5460Å total extinction values also have been reported

Table 3. Summary of cytophotometric results for the two groups of bulls.

Parameter	Lowered fertility			Normal fertility		
	No. bulls	Mean per head	± s. e. m.	No. bulls	Mean per head	± s. e. m.
Total ext. at 2650Å (μ^2)	8	6.70	0.03	7	6.62	0.02
Total ext. at 2800Å (μ^2)	8	4.61	0.04	7	4.67	0.02
Total ext. at 5460Å (μ^2)	8	4.53	0.22	7	3.58	0.32
Surface integr. o.p.d. ($10^{-12}cm^3$)	8	1.51	0.01	7	1.49	0.01
Optical area (μ^2)	5	41.3	0.9	7	41.4	1.3

for epididymal spermatozoa from bulls with lowered fertility. This previous study utilized some of the bulls in the present study. Both the previous and present studies show an increase in Feulgen stainability similar to that reported by *Parez et al.* (1960); but contrary to these investigators, the increase has not been interpreted as an increase in DNA content.

As stated above, the difference between the mean values of the two groups for u.v. absorption measurements is non-significant ($P > 0.05$) and shows that the ejaculated spermatozoa analyzed in this study contain the same mean amount of DNA regardless of the fertility status of the bull producing them. The fact that the difference between the mean integrated o.p.d. values (Table 3) is non-significant ($P > 0.05$) demonstrates that the spermatozoal head mean total dry mass does not differ between the two groups of bulls. This is an additional indication that the mean amount of DNA does not differ between the two groups and that the observed difference between the two mean 5460Å total extinction values for Feulgen stained spermatozoa is not due to a difference in the mean DNA amount.

Qualitative differences in the basic nuclear protein bound to the DNA may offer a source of explanation for the observed difference between the two groups in respect to the Feulgen stainability of spermatozoa. The background to this statement is that, normally during spermiogenesis, changes in the com-

position and binding of the basic nuclear protein component of the deoxyribonucleoprotein complex are found to occur concomitantly with a marked reduction in the Feulgen reactivity of maturing spermatids despite constant amounts of DNA (*Gledhill et al.* 1966a). This reduction in Feulgen reactivity is significantly *less* pronounced in both epididymal and ejaculated spermatozoa from bulls with lowered fertility than in spermatozoa from normally fertile bulls (*Gledhill* 1966b). In an investigation of the relationship between spermatozoal basic nuclear protein and fertility status, *Gledhill et al.* (1966b) recently found that, in a bull with severely reduced fertility of clinically unknown origin, the changes in nuclear protein which normally occur during spermiogenesis were markedly altered. Thus, there is reason to suspect that the basic nuclear protein of the spermatozoa may influence the fertility of a bull.

The reasons for the infertility of each of the bulls in the present lowered fertility group most likely vary greatly and it would indeed be surprising to find that each of these eight animals was infertile for the same reason. The lowered fertility group is therefore best thought of as being heterogeneous as far as cause of infertility is concerned. The considerable overlapping of mean 5460Å total extinction values for spermatozoa from individual bulls between the two groups of bulls (Table 2) is to be expected since many of the bulls in the lowered fertility group probably produced spermatozoa completely normal in respect to deoxyribonucleoprotein.

It has been reported (*Leuchtenberger et al.* 1956) that the variation in mean amount of spermatozoal DNA per bull is greater among infertile bulls than it is among normally fertile bulls. Within each cytophotometric parameter in the present study, the variances of bull means within the two groups were tested for equality (*Dixon & Massey* 1957). At the 5 per cent level of significance the variances were equal indicating that the same degree of variation, be it biological, methodological or a combination of both, is found for the mean DNA content, the mean total dry mass and the mean optical area of spermatozoal heads regardless of whether they were produced by the normal bulls or the bulls with lowered fertility.

CONCLUSIONS

Based on the cytophotometrically obtained data in this study, the following conclusions are made.

1. Morphologically normal ejaculated spermatozoal heads from the eight bulls with lowered fertility contain the same mean amount of DNA and have the same mean total dry mass and mean optical area as do spermatozoal heads from the seven normally fertile bulls.

2. The occurrence of a higher mean value for Feulgen stained heads from the bulls with lowered fertility in comparison to the mean value obtained for spermatozoa from the normally fertile bulls is probably due to several of the animals with lowered fertility having a spermatozoal basic nuclear protein that is in some way atypical.

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SUMMARY

Microspectrophotometric, microinterferometric and microplanimetric techniques were used to determine the DNA content, the total dry mass and the optical area of morphologically normal ejaculated spermatozoal heads from eight bulls with lowered fertility and seven bulls with normal fertility. No difference in mean amount of DNA, in mean total dry mass or in mean optical area was found to exist between spermatozoal heads from the bulls in the two groups. In comparison to the mean value for the Feulgen stained heads from the normal bulls, a higher mean value was found for spermatozoa from bulls with lowered fertility. It was suggested that this divergence in mean values might be a reflection of qualitative differences in the basic nuclear protein bound to the DNA which affected the Feulgen stainability of spermatozoa from several of the bulls with lowered fertility. None of the cases of infertility in this study could be attributed to differing amounts of spermatozoal DNA but some of the cases might have been caused by an altered basic nuclear protein in the spermatozoa.

ZUSAMMENFASSUNG

Untersuchungen über den DNA-Gehalt, das Trockengewicht und die Fläche ejakulierter Spermatozoenköpfe von Bullen mit normaler und herabgesetzter Fertilität.

Mikrospektrophotometrische, mikrointerferometrische und mikroplanimetrische Methoden wurden angewandt, um den DNA-Gehalt, das Trockengewicht und die Fläche morphologisch normaler ejakulierter Spermatozoenköpfe von acht Bullen mit herabgesetzter und sieben Bullen mit normaler Fertilität zu untersuchen. Es konnte kein Unterschied zwischen den Mittelwerten für den Gehalt an DNA, das Trockengewicht und die Fläche von Spermatozoenköpfen der Bullen beider Gruppen gefunden werden. Verglichen mit dem Mittelwerte für die nach Feulgen gefärbten Spermatozoenköpfe normaler Bullen wurde ein höherer Durchschnittswert für Spermatozoenköpfe für Bullen mit herabgesetzter Fertilität gefunden. Es wird angenommen, dass diese Divergenz in den Mittelwerten qualitative Unterschiede in den basischen, an die DNA gebundenen Kernproteinen wiederspiegelt. Solch ein Unterschied könnte die Feulgen-Färbbarkeit der Spermatozoen von einigen der Bullen mit herabgesetzter Fertilität angreifen. Keiner der in dieser Arbeit untersuchten Infertilitätsfälle konnte auf unterschiedlichen Gehalt der Spermatozoen an DNA zurückgeführt werden, aber einige Fälle sind möglicherweise durch verändertes basisches Kernprotein verursacht.

SAMMANFATTNING

Undersökningar över DNA-mängd, torrvikt och cellyta av ejakulerade spermiehuvuden från tjurar med normal och nedsatt fertilitet.

Mikrospektrofotometriska, mikrointerferometriska och mikroplanimetriska metoder användes för att bestämma DNA-mängd, torrvikt och cellyta av morfologiskt normala huvuden hos ejakulerade spermier från åtta tjurar med nedsatt fertilitet samt från sju tjurar med normal fertilitet. Inga skillnader i den genomsnittliga mängden DNA, torrvikt och cellyta kunde påvisas i spermiehuvudena från de två grupperna. I jämförelse med medelvärdet för de Feulgenfärgade huvudena från de normala tjurarna, befanns medelvärdet för spermierna från tjurarna med nedsatt fertilitet vara högre. Denna skillnad i medelvärde ansågs kunna återspegla kvalitativa skillnader i de DNA-bundna basiska kärnproteinerna, vilket i sin tur påverkade spermiernas Feulgenfärgbarhet hos flera av tjurarna med nedsatt fertilitet. I inget av infertilitetsfallen i denna undersökning berodde skillnaderna på olikheter i mängd DNA utan ansågs snarare i flera av fallen orsakas av förändringar i spermiernas basiska kärnproteiner.

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