MECHANISM OF THE REDUCTION IN DNA CONTENT IN SPERM CELLS PRESERVED IN VITRO

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The DNA content in viable sperm cells preserved in vitro remains practically the same as in the spermatozoa of freshly obtained sperm no matter how long they are kept. The DNA level in dying or dead spermatozoa falls.

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There are reports in the literature [7, 8] of a decrease in the DNA content in sperm cells when preserved in vitro. According to some information [7], the decrease in the DNA content in spermatozoa during preservation for 5 days in a yolk-citrate medium at 5° may reach 30%. Under these conditions the sperm still maintains relatively high fertility. The results of these investigations have been widely cited as contradicting the familiar view of the constancy of the DNA content in cells. No doubts have been expressed about the reliability of this work. However, as we have pointed out previously [1-3], determination of the DNA content in cells, for various reasons connected with modern methods used to study this problem, reflects only the mean DNA content in cells irrespective of their functional state. This leads to unjustified conclusions concerning the possibility of a true decrease in the DNA level in all cells of the studied population during exposure to a particular harmful agent. In previous studies [4] by the method of cytophotometry of spermatozoa stained by Feulgen's method, we showed that the observed decrease in the DNA content in the spermatozoa of preserved sperm is determined by the decrease in the DNA content in the nuclei of only those cells whose function is disturbed. In those cells which have retained their viability, the DNA content remains unchanged during preservation of the sperm. This conclusion of ours has recently been confirmed by other workers [5].

The object of the present investigation was to verify this principle, but using a different method of determination of the DNA content in the cells—the method of ultraviolet cytospectrophotometry (UVC). It will be recalled that some workers [6], using the UVC method, found no difference in the DNA content in the heads of normal and abnormal (in shape) bovine spermatozoa.

EXPERIMENTAL METHOD

Sperm cells of boars were investigated. The freshly obtained sperm was diluted (1:1) with 6% glucose solution containing antibiotics and kept at 15°. After preservation of the sperm for 5 days the viable (motile) spermatozoa were separated from the nonviable (nonmotile, staining with eosin) by the filtration method of Paré and Rouiller. Fused quartz balls 200-300 μ in diameter were used as the filter. By this method, a fraction (the filtrate) containing about 90% of viable cells could be obtained from sperm kept for 5 days and containing only about 30% of motile spermatozoa not staining with eosin. The DNA content in the cells of the filtrate, as in other samples of sperm, was determined by the UVC method. Since the dimensions of the sperm cells vary during keeping, the area of the heads of the spermatozoa was also determined by calculation by a special formula [9] from measurements of their length, their width at the base and the maximum width of the head.

The DNA content in the spermatozoa was determined by a photographic modification of the differential UVC method. The cells were photographed by means of an MUF-6 camera on FT-31 film at wavelengths of 254 and 280 mμ.

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TABLE 1. Comparative Characteristics of Principal Indices (optical density, area of heads, concentration and content of DNA) in Fresh Spermatozoa and Spermatozoa in Filtered and Unfiltered Sperm Kept for 5 Days

Sperm	No. of cells measured	Optical density	DNA conc. (in g/cm³)	Area of heads (in μ^2)	DNA content per cell		
					In g · 10^{-12}		in %
					M±m	P	
Freshly obtained	399	0.097	$115 \cdot 10^{-3}$	29.94	3,44±0.019	—	100.0
Unfiltered, 5 days old . . .	355	0.081	$94 \cdot 10^{-3}$	31.20	2.99±0.027	$P<0.01$	86,9
Filtered, 5 days old.	149	0.093	$111 \cdot 10^{-3}$	30.71	3,41±0.043	$P<0.5$	99.1

A water-immersion objective (58 ×) with a 0.8 numerical aperture and quartz projection camera ocular (8 ×) were used for the experiments. The images of the cells on the negative were measured on an MF-2 microphotometer by a scanning method. The area of the cell measured by the photometer was 0.25 μ^2. The optical density was calculated from the formula:

$$D_\lambda = \frac{S_c - S_n}{\gamma},$$

where S_c represents the blackening of the part of the negative free from image of the cell, S_n the blackening of the part containing the cell image, and γ the contrast factor of the photographic emulsion. To calculate the contrast factor, cells were photographed twice at the same wavelength with exposures of 5 and 10 sec. In that case

$$\gamma = \frac{S_1 - S_2}{\lg t_2 - \lg t_1} = \frac{S_1 - S_2}{\lg 2},$$

where S_1 and S_2 are the intensities of blackening of points on the negative free from image of the cell at exposures of $t_1 = 5$ sec and $t_2 = 10$ sec respectively. Since the coefficients of absorption of proteins (K^p_{254} and K^p_{280}) and the nonspecific light losses (L_{254} and L_{280}) for wavelengths of 254 and 280 mμ respectively are practically equal, the DNA was not extracted from the cells, nor were they photographed at a wavelength of 313 mμ. The optical density due to absorption of DNA was calculated as the difference between optical densities at wavelengths of 254 and 280 mμ, for with $K^p_{254} = K^p_{280}$ and with $L_{254} = L_{280}$, the DNA concentration (C) is directly proportional to the difference between these optical densities:

$$C.d\ (K^{DNA}_{254} - K^{DNA}_{280}) = D_{254} - D_{280}.$$

This equation was then used to calculate the concentration, after which it was multiplied by the volume of the head of the spermatozoa to determine the DNA content per cell. The thickness of the cell layer examined in the photometer was taken as 1 μ.

EXPERIMENTAL RESULTS

As Table 1 shows, the DNA content in spermatozoa of fresh sperm was 3.44×10^{-12} g per cell, in agreement with data in the literature. In cells of an unfiltered sample of sperm kept for 5 days the DNA content was reduced by 13.1%, somewhat less than the results obtained by other workers [7, 8] investigating spermatozoa of bovine sperm. These quantitative differences may be explained by differences in sensitivity of porcine and bovine sperm to preservation. In addition, the results cited above [7, 8] were obtained by cytophotometry of specimens stained by Feulgen's method, whereas we used to UVC method. The hyperchromic effect due to changes in secondary structure of the DNA and the formation of products of its partial hydrolysis in dying or dead cells could be reflected in these results, because in the experiments using the UVC method it could reduce slightly the percentage of decrease in the DNA content in spermatozoa kept for 5 days. However, this factor could not in any way affect the fundamental conclusion emerging from our results.

It is clear from Table 1 that the DNA content in cells of a filtered specimen of sperm kept for 5 days, in which 90% of the cells were viable, was almost the same as that in the cells of freshly obtained sperm. It can thus be concluded that the decrease in the DNA content in the spermatozoa during keeping is due to a decrease in the DNA level in dying or dead cells. The DNA content in spermatozoa remaining viable during keeping is independent of the keeping time of the sperm and remains at or almost at the same level as in cells of freshly obtained sperm.

LITERATURE CITED

1. I. I. Ivanov, O. N. Borovikova, V. G. Vladimirov, et al., in: 6th International Congress of Biochemistry, Abstracts, Vol. 1, New York (1964), p. 62.
2. I. I. Ivanov, O. N. Borovikova, V. G. Vladimirov, et al., Dokl. Akad. Nauk SSSR, 155, No. 3, 683 (1964).
3. I. I. Ivanov, O. N. Borovikova, V. G. Vladimirov, et al., in: Effect of Ionizing Radiations on Heredity [in Russian], Moscow (1966), p. 85.
4. I. I. Ivanov, N. V. Korban, and V. I. Sharobaiko, Tsitologiya, No. 10, 1294 (1967).
5. V. P. Kononov, Zhivotnovodstvo, No. 12, 66 (1967).
6. V. L. Gledhill, Acta Vet. Scand., 7, 1 (1966).
7. G. W. Salisbury, W. J. Birge, L. de la Torre, et al., J. Biophys. Biochem. Cytol,, 10, 353 (1961).
8. W. Summerhill and D. Olds, J. Dairy Sci., 44, 548 (1961).
9. C. Van Duijn, Jr., Mikroskopie, 15, 142 (1960).