Use of dithiothreitol for improved visibility of the F body in human Y-bearing spermatozoa

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Summary. Dithiothreitol was used to decondense the nuclear chromatin before staining human spermatozoa with quinacrine for the F body present in Y-bearing spermatozoa. In unseparated fractions and those enriched in X- or Y-bearing spermatozoa, the number of spermatozoa with F bodies increased by 2–9% after dithiothreitol treatment.

Introduction

Our studies on the separation of X- and Y-bearing spermatozoa in human semen (Hegde, Shastry & Rao, 1977; Shastry, Hegde & Rao, 1977) have indicated that the method developed by Barlow & Vosa (1970) is reliable for the detection of the F body present in the Y-spermatozoa, although the percentage of Y-spermatozoa detected was lower than the expected value of 50% in different semen samples. The reasons for the lower percentage could be due to fast fading of the fluorescence, a low uptake of the dye which would decrease the visibility of the F body, or the F body might be situated in the interior portion of the compact nuclear chromatin and therefore not be accessible to quinacrine staining (Barlow & Vosa, 1970).

The last possibility can be investigated by treating spermatozoa with a reagent which decondenses the nuclear chromatin and thus exposes the F body. Dithiothreitol has been used with or without sodium dodecyl sulphate (SDS) to decondense nuclear chromatin (Bedford, Bent & Calvin, 1973). In sperm immunology this reagent is used to expose nuclear antigens which are otherwise not seen by immunofluorescence (Kolk, Samuel & Rumke, 1974; Tung, 1975). Dithiothreitol was therefore tested to see whether a more accurate identification of the proportion of Y-bearing human spermatozoa could be obtained.

Materials and Methods

Semen samples were obtained by masturbation from fertile men and processed separately (Shastry *et al.*, 1977) to obtain enriched fractions of X- and Y-spermatozoa. The washed spermatozoa were layered onto the Ficoll–sodium metrizoate column of density 1.08 and used directly, without layering again, on Ficoll of density of 1.04 as described earlier. The spermatozoa examined were in samples from the unseparated semen, from the interface and from the sediment. Each sample was divided into two fractions. One fraction was treated with dithiothreitol and the other was the untreated control which was otherwise processed similarly.

A 4 mM solution of dithiothreitol (Sigma) was prepared in a Tris–HCl buffer, pH 9, and an equal volume of this solution was added to the sperm suspension. The optimum time required for the desired degree of decondensation of the nuclear chromatin was found to be 20 min. The spermatozoa were incubated with dithiothreitol for 10 min and centrifuged immediately for 10 min at 100 g; no reagent was used to stop the reaction, but if spermatozoa were treated for more than 20 min, they tended to disintegrate into a jelly-like mass. The supernatant containing the dithiothreitol was removed and the cells were suspended in a 0.15 M-phosphate-buffered saline, pH 7.2. Uniform smears were made on clean slides and were stained for F bodies by the method of Barlow & Vosa (1970).

The stained slides were examined in an 'Olympus' fluorescence microscope: 10 fields and not less than 50 nuclei were scored for each sample.

Results

Treatment of human spermatozoa with dithiothreitol results in swelling of the nuclei and the entire cell and so it was not possible to assess the F body visibility in untreated and treated samples without being aware of the treatment. Every result was therefore cross-checked by another observer. As shown in Table 1 the F bodies in the samples varied from 35 to 46%. When the unseparated semen samples were treated with dithiothreitol there was an increased percentage of observed F bodies. This increase was 7–9% in samples in which the F body percentage was lower than 40%, and about 2% when the F body percentage was about 45%. This change in visibility of the F body was obtained with enriched fractions of X- and Y-spermatozoa; the increased percentage of F bodies after treatment with dithiothreitol was about 3% for spermatozoa in the interface and sediment layers (Table 1).

Table 1. The effect of dithiothreitol (DTT) on the visibility of the F body in human Y-bearing spermatozoa

Sample no.	Y spermatozoa detected by F bodies					
	Unseparated fraction		Interface fraction		Sediment fraction	
	Untreated	DTT treated	Untreated	DTT treated	Untreated	DTT treated
1	35.11	44.21	26.39	26.51	67.14	73.53
2	37.00	45.80	25.58	28.98	66.66	70.50
3	41.77	43.18	26.67	30.00	63.64	68.64
4	39.76	49.77	26.25	32.65	69.86	70.27
5	35.00	42.00	29.17	32.17	68.00	69.51
6	45.68	47.44	28.17	28.80	72.50	73.24
7	45.26	47.46	28.38	33.72	63.95	71.25
8	42.06	45.68	23.29	28.29	68.93	74.69
Mean	40.21	45.69*	26.73	30.14*	67.59	71.45*
s.d.	4.224	2.530	1.860	2.478	2.955	2.138
s.e.m.	1.490	0.900	0.660	0.880	1.040	0.760

* These values were significantly different from that for the corresponding untreated sample, $P < 0.01$ (paired t test).

The difference in the visibility of the F body in the different fractions before and after treatment with dithiothreitol was statistically significant ($P < 0.01$), indicating the usefulness of this reagent for improving the visibility of the F body.

Discussion

The mean value for Y-chromosomes was less than 40%, as reported by Barlow & Vosa (1970), and the improved visibility after dithiothreitol treatment clearly indicates the value of this modification for detecting those F bodies lying in the interior of the nuclear chromatin by increasing the uptake of the dye into the swollen nucleus. Dithiothreitol acts irreversibly and is effective in low concentration. With spermatozoa it decondenses the nuclear chromatin and results in sperm swelling. The decondensation is brought about by the cleavage of S–S bonds present in the nucleoproteins (Bedford *et al.*, 1973). We noted that dithiothreitol-treated human spermatozoa vary in the degree of decondensation achieved, as reported by Bedford *et al.* (1973). The implication of this variation is not known. Comparison of the swelling rate of nuclear chromatin of spermatozoa in the presence of seminal

plasma with that of washed aliquots showed that substances present in contaminating seminal plasma were able to depress the rate of the nuclear swelling reaction (Bedford *et al.*, 1973). In our studies, we therefore eliminated the interference of seminal plasma by treating washed spermatozoa with dithiothreitol. The use of dithiothreitol is recommended only to get a better evaluation of the percentage of Y-bearing spermatozoa. F bodies are visualized very clearly after treatment of spermatozoa with dithiothreitol and can be detected without difficulty by inexperienced workers.

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