

INDUCED NUCLEAR METACHROMASY EVALUATED IN SPERMATOZOA OF "PÉ-DURO" BULLS*

C.M.C. Britto^{1,2} and M.L.S. Mello¹

ABSTRACT

The phenomenon of induced nuclear metachromasy was investigated in sperm cells from "pé-duro" bulls compared to those of specimens from an elite herd. The aim was to detect chemical anomalies at the level of DNA-protein complexes which affect the fertilizing capacity of the spermatozoa of these animals. Thirty percent of the "pé-duro" specimens showed high percentages of spermatozoa with nuclear metachromasy besides other characteristics which do not recommend preservation of their semen. The other "pé-duro" bulls showed low percentages of metachromatic sperm cell nuclei. However, not all of them appear to be indicated as donors of semen for preservation. Some of them (nearly 40% of the sample) present oligozoospermy and significant percentages of morphological abnormalities in the sperm cells. The search for induced nuclear metachromasy in bull spermatozoa must thus be accompanied by a morphological analysis of the sperm cells and determination of the spermatozoal concentration in the ejaculate.

INTRODUCTION

The nuclear basophilia which appears in cell preparations stained with a toluidine blue solution at pH 4.0 is promoted by the presence of nucleic acids, as the dye molecules bind electrostatically to available DNA and RNA phosphate groups (Lison, 1960; Miura and Ohba, 1967). In preparations stained with toluidine blue the

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¹ Departamento de Biologia Celular, Instituto de Biologia, UNICAMP, 13081 Campinas, SP, Brasil. Send correspondence to M.L.S.M.

² Permanent address: Departamento de Biologia, Universidade Federal do Piauí, 64000 Teresina, PI, Brasil.

phenomenon of basophilia is called metachromasy when the absorption maximum of the stained substrate is positioned at $\lambda = 540\text{-}550\text{ nm}$ (λ -metachromasy, violet color) or $\lambda = 570\text{-}580\text{ nm}$ (β -metachromasy, bluish-violet color) (Lison, 1960; Toepfer, 1970).

Bull sperm cell nuclei do not exhibit metachromasy after treatment with a toluidine blue solution at pH 4.0, except when their protamine is released experimentally from the DNA-protein complex (Mello, 1982). After acid hydrolysis, however, nuclear metachromasy is detected in sperm cell nuclei suggested to be bearing an altered (immature?) basic nuclear protein attached to the DNA. This phenomenon is not necessarily associated with changes in germ cell morphology (Mello, 1982). The induced nuclear metachromasy in spermatozoa without head defects is diagnosed at higher frequency in subfertile animals (Mello, 1982).

In Brazil there has been a recent scientific effort to preserve the germplasm of "pé-duro" bulls, as these animals, though well adapted to the drought conditions of the hinterlands of the states of Piauí and Maranhão, are condemned to extinction due to indiscriminate slaughter (Carvalho and Pereira, 1981; Carvalho, 1983, 1984).

The spermatozoa of "pé-duro" bulls have been investigated in terms of morphological characteristics and Feulgen-DNA content, which could indicate that their fertilizing capacity is jeopardized (Britto and Mello, 1988; Britto *et al.*, 1988). Based on these criteria, at least 30% of the analyzed specimens have been recommended for semen preservation for artificial insemination.

As part of an investigation of nuclear cytochemical characteristics of the spermatozoa of these animals, in the present study the phenomenon of induced nuclear metachromasy was ascertained in spermatozoa from semen samples previously analyzed by the methodological procedures mentioned above (Britto and Mello, 1988; Britto *et al.*, 1988).

MATERIAL AND METHODS

Smears of semen samples from ten "pé-duro" bulls and from ten bulls of an elite herd (control) were prepared. Bull origin has been reported in a previous publication (Britto and Mello, 1988).

The preparations were fixed in ethanol-acetic acid (3:1) for 1 minute, rapidly rinsed in 70% ethanol and air dried. Acid hydrolysis with 4N HCl was carried out at 25°C for 10, 15 and 20 min prior to staining with toluidine blue, pH 4.0, for 15 minutes (Mello, 1982). The smears were then rapidly rinsed in distilled water, air dried, cleared in xylene for 15 minutes and mounted in Canada balsam.

Slides were examined with a Zeiss Universal microscope to determine the frequency of metachromatic nuclei. First, each smear was screened for morphologically normal and abnormal nuclear populations by determining the number of nuclei

present in 520 sequential microscopic fields per slide. This observation was performed in preparations subjected to the Feulgen reaction since non-metachromatic nuclei are very hard to discriminate in toluidine blue-treated smears. Considering that the volume of semen dropped on slides and the smearing conditions were the same for the various preparations obtained from the same ejaculate, it is assumed that the number of spermatozoa in smears subjected to the Feulgen reaction was practically equal to that in smears treated with toluidine blue from the same ejaculate. The second step consisted of detecting the total number of metachromatic nuclei in 520 sequential microscopic fields per slide in preparations treated with toluidine blue.

RESULTS

The number of metachromatic nuclei detected in spermatozoa with or without head defects from "pé-duro" bulls and from an elite herd after different hydrolysis times is given in Tables I and II.

The frequency of normally shaped spermatozoa with metachromatic nuclei in elite animals was practically null. However, for spermatozoa with abnormally shaped

Table I - Percentage of metachromatic nuclei for spermatozoa of elite bulls.

Animal code	Total no of nuclei		Total metachromatic nuclei (%)					
			Hydrolysis times (minutes)					
			10		15		20	
NM	AM	NM	AM	NM	AM	NM	AM	
431*	9640	928	0.0	0.2	0.1	1.3	0.1	1.6
762*	19600	700	0.0	0.4	0.0	0.7	0.1	1.1
770*	9860	268	0.0	0.4	0.0	1.1	0.1	1.5
437*	19890	500	0.0	0.2	0.0	0.6	0.0	0.8
855*	19180	1800	0.1	0.9	0.1	1.0	0.1	1.6
829**	9800	440	0.0	0.2	0.0	0.7	0.0	0.9
730**	19540	216	0.0	0.5	0.0	0.5	0.0	0.9
616**	12550	1780	0.0	0.6	0.0	1.1	0.1	1.5
280**	10116	189	0.0	0.5	0.0	0.5	0.0	0.5
719**	10788	125	0.0	0.8	0.0	0.8	0.1	1.6

AM = abnormal morphology; NM = normal morphology; **Bos taurus*; ***Bos indicus*.

Table II - Percentage of metachromatic nuclei in spermatozoa of "pê-duro" bulls.

Animal code	Total no. of nuclei		Total metachromatic nuclei (%)					
			Hydrolysis times (minutes)					
	NM	AM	10		15		20	
			NM	AM	NM	AM	NM	AM
104	9908	1480	0.2	0.5	0.5	0.7	0.8	2.3
115	4800	120	0.0	0.8	0.0	0.8	0.1	1.7
118	17580	1720	0.1	0.7	0.2	1.1	0.5	2.1
4	16700	180	0.0	0.6	0.0	0.6	0.1	0.6
5	17928	230	0.0	0.4	0.1	0.4	0.6	0.9
6	1080	85	0.4	1.2	0.5	1.2	0.6	2.4
7	18340	456	0.1	0.2	0.2	0.5	0.3	0.9
8	1100	59	1.0	3.4	1.9	5.1	5.7	13.6
9	1460	184	1.6	1.6	1.9	2.7	2.3	4.9
10*	9001	629	1.0	1.9	2.1	2.7	2.2	4.5

AM = abnormal morphology; NM = normal morphology; * = half breed.

heads the number of metachromatic nuclei increased with advancing hydrolysis times, attaining 1.5% for 50% of the animals (especially *Bos taurus*) after a 20-minute hydrolysis period (Table I).

On the other hand, a large number of metachromatic nuclei was detected in normally or abnormally shaped spermatozoa of "pê-duro" bulls nos. 8, 9 and 10, especially at the hydrolysis time of 20 minutes (Table II). Bulls nos. 115 and 6, previously demonstrated to have oligozoospermy (Britto and Mello, 1988), exhibited metachromasy in 1.7% of their abnormally shaped spermatozoal nuclei after the 20-minute hydrolysis treatment (Table II). In spermatozoa from specimens nos. 104 and 118, which exhibited a large number of abnormally shaped heads, just like those of specimens nos. 8, 9 and 10 (Britto and Mello, 1988), the frequency of metachromatic nuclei was 2.1-2.3%, also after the 20-minute hydrolysis treatment (Table II). However, even specimens nos. 104, 118, 115 and 6 did not show more than 1.0% metachromatic nuclei in normally shaped spermatozoa.

DISCUSSION

The "pê-duro" bulls which presented nuclear metachromasy in a relatively

high percentage of their normally shaped spermatozoa also had 11.2 to 14.3% lower than average Feulgen-DNA values (Britto *et al.*, 1988). In addition, these specimens exhibited high percentages of spermatozoal head defects (Britto and Mello, 1988). Possibly, the same abnormality which affected the spermatozoal response to the Feulgen reaction made the spermatozoal DNA available to toluidine blue binding, resulting in nuclear metachromasy. Both cytochemical reactions involve a hydrolysis step which differently affects the various DNA-protein complexes. It is assumed that a not so intimate interaction of nuclear proteins with DNA occurs in those spermatozoa with metachromatic nuclear staining.

On the other hand, no metachromasy was detected in the sperm cell nuclei of the other "pé-duro" specimens and of elite bull no. 616, though these animals produced spermatozoa with a high percentage of head defects (Britto *et al.*, 1988). Anyway, the data on spermatozoal head defects are sufficient to exclude these animals as semen donors if the ejaculate characteristics are maintained even under improved zoo-technical situations.

It is concluded that subfertility diagnosed on the basis of oligozoospermy and high percentages of spermatozoal head defects is not necessarily accompanied by chemical changes affecting the DNA-protein complex or normally shaped spermatozoa. On the other hand, when these chemical changes are present, the semen also shows significant percentages of spermatozoal head defects.

Association of morphological and cytochemical analyses thus appears to be recommended for quality discrimination in the semen of "pé-duro" bulls. Among the cytochemical methods, induced nuclear metachromasy is preferable to detection of Feulgen-DNA values, since the latter is more complex and involves expensive equipment requiring costly maintenance. The search for nuclear metachromasy is a simple task performed even with an ordinary microscope. For the investigation of induced metachromasy in the nuclei of bull spermatozoa a hydrolysis time of 20 minutes proved to be the most efficient.

Considering the sum of data obtained for "pé-duro" bulls, it is thus concluded that the quality of semen of at least 30% of the investigated specimens is as good for preservation as that of most elite bulls. This low profit percentage is not an unexpected fact. A cattle raising census (Censo Apropetário do Piauí, 1980) has reported 40% reproductivity in the Piauí state herd. By improving the nutrition and health management of these animals, their zootechnical level may increase and more specimens could be available as semen donors.

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RESUMO

O fenômeno de metacromasia nuclear induzida foi investigado em espermatozoides de touros "pé-duros" comparados aos de animais de elite. O objetivo foi detectar níveis de defeitos químicos do complexo DNA-proteína que comprometessem a capacidade fertilizadora dos espermatozoides nesses animais. 30% dos animais "pés-duros" investigados apresentaram altos percentuais de espermatozoides com metacromasia nuclear, além de outras características que desaconselham a preservação do seu sêmen. O restante dos animais "pés-duros" apresentou percentuais baixos de espermatozoides exibindo metacromasia nuclear induzida. No entanto, nem todos estes são aconselhados como doadores de sêmen para preservação. Alguns dos mesmos ($\cong 40\%$ da amostragem) apresentam oligozoospermia e espermatozoides com altos percentuais de anormalidades morfológicas. A busca de metacromasia nuclear induzida deve, pois, ser associada à análise morfológica e de concentração de espermatozoides no ejaculado, quando se pretende discriminar anomalias em sêmen de touros.

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