COMPARATIVE STUDY BETWEEN PHASE CONTRAST AND DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPIES FOR EVALUATION OF FROZEN BULL SEMEN

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BARNABE, V.H.; BARNABE, R.C.; VISINTIN, J.A.; VIANA, W. G.; CASAGRANDE, J.F.; ALMEIDA, C.A. Comparative study between phase contrast and differential interference contrast microscopies for evaluation of frozen bull semen. **Rev.Fac. Med.vet.Zootec.Univ.S.Paulo, 18(1): 55-59, 1981. 1981.**

SUMMARY: Two hundred ampules of frozen bull semen were evaluated for per cent acrosomal pathology and major and minor defects of spermatozoa. The ampules referred to 4 groups of 50 each, corresponding to semen frozen in 1975, 1976, 1977 and 1978. Semen examinations were made after thawing and after being placed in a 38° C water bath for 5 hours (Slow Thermoresistance Test) or in a 45° C water bath for 1 hour (Quick Thermoresistance Test). Analysis of variance showed highly significant differences ($P < .01$) between phase-contrast and differential interference contrast microscopies for evaluation of acrosomal pathology and major defects of spermatozoa. For minor defects analysis of variance did not show statistical differences between the two technics employed.

UNITERMS: Differential interference contrast microscopy*; Phase-contrast microscopy*; Frozen bull semen evaluation*

INTRODUCTION

Several studies have shown that impaired fertility in the bull may be related to morphologic defects in spermatozoa. Thus, evaluation of spermatozoal morphologic features is an important aid in assessing a bull's breeding soundness. Spermatozoal morphologic features have generally been evaluated in stained seminal smears. Although procedures for preparing stained smears may be detrimental to spermatozoa integrity[5].

Phase contrast and differential interference contrast microscopies make it possible to evaluate spermatozoa morphologic features in wet preparations of semen, with buffered formol saline or 0.2% glutaraldehyde in phosphate-buffered saline. This last fixation procedure allows the transport of semen samples preventing cellular injuries[9] and the possibility of storage up to 29 days[6].

Resolution of differential interference contrast is improved over that of phase contrast microscope because interference halos are greatly minimized[10].

Differential interference contrast microscope has been used for the study of correlations between spermatozoal abnormalities and fertility[10] and for routine evaluation of semen, including all the ejaculations of bulls in service[1,2,4,7,11].

The objective in the present investigation was to compare phase contrast and differential interference contrast methods, regarding acrosome evaluation and major and minor defects[3] in frozen semen of bulls.

MATERIAL AND METHOD

There were studied 200 ampules of frozen semen from 10 bulls, donors in an Artificial Insemination Centre placed in Barretos, São Paulo State, Brazil. The ampules referred to four groups of 50 each, corresponding to semen frozen in 1975, 1976, 1977 and 1978.

Fixation with buffered formol saline or buffered glutaraldehyde was accomplished by pipetting a drop of semen into 4.5 ml vials containing 2 ml of fixative. Wet mounts were prepared by placing a drop of fixed semen on the center of clean slides under 22 by 30 mm coverslips luted by nail varnish.

Semen evaluations were made after thawing and after being placed in a 38°C water bath for 5 hours (Slow Thermoresistance Test) or in a 45°C water bath for 1 hour (Quick Thermoresistance Test)[1].

Buffered formol saline material was examined under 1000 x magnifications phase contrast microscope in oil immersion.

Differential interference contrast microscopy was used to evaluate wet preparations in buffered glutaraldehyde at 1250 x magnifications in oil immersion.

With each method, 200 spermatozoa were evaluated per slide with results give in percentage. Classification into major and minor defects[3] was adopted, besides acrosome evaluation.

Data were analysed by analysis of variance[8]. Differences between treatments were compared using F test, fixing

the rejection level to nullity hipothesis in 0.01%.

RESULTS AND DISCUSSION

As it may be seen in Table 1, higher ($P < 0.01$) percentages of alterations of the acrosomal cap and major defects were obtained on semen samples fixed in buffered glutaraldehyde examined under differential interference contrast microscope than on buffered formol saline using phase contrast microscope. Relatively to minor defects both methods did not differ for interpretation of sperm abnormalities. These features have been shown in post thawing semen and even as after incubations tests to 38°C or 45°C.

Particularly in relation to acrosome and head abnormalities whose interpretation requires more carefulness, more defects were counted in differential interference contrast microscope than in phase contrast, even though it was the same original semen sample. High magnifications can be obtained with phase contrast. However, due to interference halos around sperm[10], resolution is not quite as good as that obtained with differential interference contrast microscope[1,2,4,7,11]. This technic provides examination with an excellent method for directly examining sperm samples for abnormalities with a high degree of precision, what has greatly enhanced the quality control program of frozen semen.

Since status of spermatozoa fixed in glutaraldehyde is not affected by transport[9] or storage time up to at least 29 days[6], seminal samples can be fixed and shipped for evaluation to laboratories where differential interference contrast microscopy is available.

Alterations of the acrosomal cap more frequently found in our samples included since loss of apical ridge and swelling of anterior acrosomal cap with formation of equatorial segment to deterioration and loss of anterior acrosomal cap.

Major defects included predominantly abnormal heads, sinuous mid-piece and some proximal droplets, while minor defects were characterized chiefly by coiled tails and loose haeds.

In Figure 1, obtained in phase contrast microscope, characteristics halos around sperm can be observed. On the other hand, in Figure 2, obtained in differential interference contrast microscope, the image produced results in sperm appearing as though the light was originating from the side, giving a clear-cut appearance.

Evaluation of acrosomal alterations accompanying sperm aging or injury as well as sperm morphology counts can be performed in differential interference contrast microscope without staining of semen smears. This not only has the advantage of saving time in slide preparation, but reduces chances of artifacts often produced in killed-stained preparations[5].

CONCLUSIONS

The comparative study between phase and differential interference constrast microscopies for evaluation of frozen bull semen after thawinh and after incubation tests has shown: 1) clear superiority of differential interference contrast over phase contrast microscopy for examination of acrosomal pathology and major defects of sperm; 2) no significant difference between the two methods for examination of minor defects.

BARNABE, V.H.; BARNABE, R.C.; VISINTIN, J.A.; VIANA, W. G.; CASAGRANDE, J.F.; ALMEIDA, C.A. Estudo comparativo entre as microscopias de contraste de fase e contraste de interferência diferencial para análise de sêmen congelado de bovinos. **Rev.Fac.Med.vet.Zootec.Univ.S.Paulo, 18**(1): 55-59, 1981.

RESUMO: Duzentas ampolas de sêmen de bovinos, constituindo 4 grupos de 50, correspondentes a congelamentos efetuados, respectivamente, nos anos de 1975, 1976, 1977 e 1978, foram estudadas para avaliação da porcentagem de patologia do acrossomo e de defeitos maiores e menores. Os exames foram realizados após o descongelamento e após submissão às provas rápida (1 hora a 45° C) e lenta (5 horas a 38° C) de termo resistência, em microscopia de contraste de fase e em microscopia de contraste de interferência diferencial. Os resultados das análises de variância mostraram haver diferença estatística, altamente significante ($P < 0,01$), a favor da microscopia de contraste de interferência diferencial para patologia do acrossomo e defeitos maiores, o mesmo não ocorrendo em relação aos defeitos menores.

UNITERMOS: Microscopia de contraste de interferência diferencial*; Microscopia de contraste de fase*; Avaliação de sêmen congelado de touros*; Estudo comparativo*.

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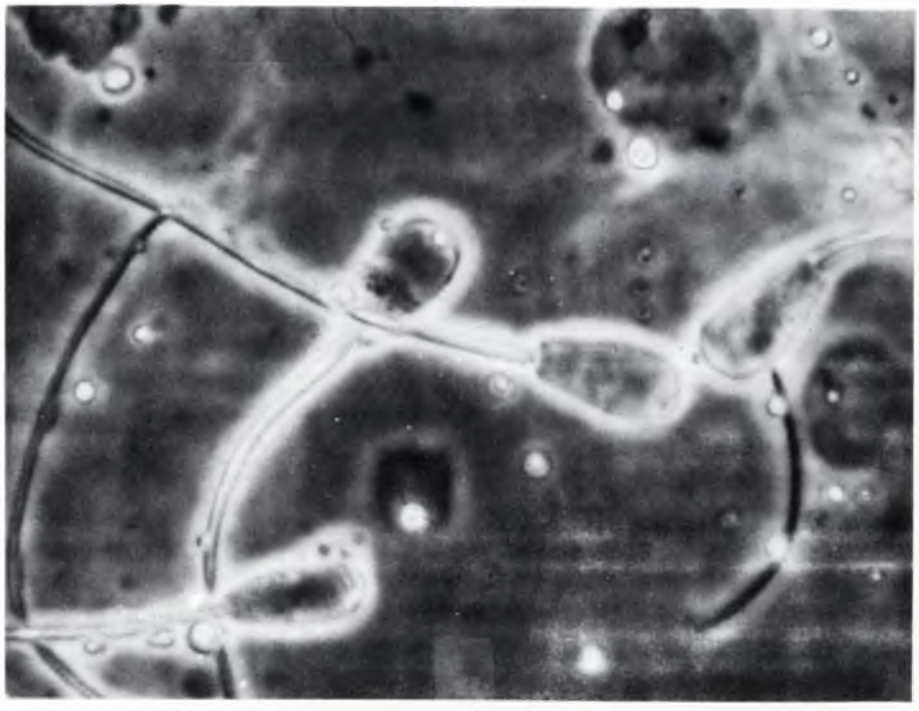

Figure 1 - Phase-contrast microscopy. Bull spermatozoa. 1000 X

Figure 2 - Differential interference contrast microscopy. Bull spermatozoa. 1250 X

Table 1 – Analysis of variance and "F" test of data from morphologics characters of spermatozoa in frozen bulls semen, in phase-contrast and differential interference contrast microscopies. S. Paulo, 1979.

SPERMATIC PATHOLOGY	YEAR OF FREEZING	AVERAGE AFTER THAWING C	I	F	AVERAGE AFTER Q.T.T. C	I	F	AVERAGE AFTER S.T.T. C	I	F
Acrosomal pathology	1975	3,54 ± 0,12	6,12 ± 0,17	149,9**	5,65 ± 0,24	7,82 ± 0,28	32,6**	7,14 ± 0,24	10,29 ± 0,24	85,5**
	1976	3,19 ± 0,14	5,17 ± 0,19	64,0**	4,63 ± 0,13	7,20 ± 0,20	111,5**	6,30 ± 0,18	9,76 ± 0,22	139,2**
	1977	2,92 ± 0,15	4,62 ± 0,17	52,7**	4,76 ± 0,17	7,00 ± 0,23	48,3**	6,11 ± 0,23	9,34 ± 0,43	43,3**
	1978	1,48 ± 0,19	2,92 ± 0,20	26,4**	5,63 ± 0,26	9,24 ± 0,46	45,3**	7,59 ± 0,37	12,83 ± 0,65	48,3**
Major defects	1975	2,48 ± 0,15	4,14 ± 0,25	32,1**	3,07 ± 0,16	4,71 ± 0,21	35,3**	3,44 ± 0,13	5,06 ± 0,18	50,4**
	1976	1,63 ± 0,10	3,08 ± 0,16	52,2**	2,59 ± 0,13	3,91 ± 0,16	39,9**	2,43 ± 0,17	4,45 ± 0,21	55,1**
	1977	1,67 ± 0,14	2,90 ± 0,19	25,5**	2,42 ± 0,17	4,15 ± 0,23	36,4**	2,79 ± 0,18	4,37 ± 0,21	31,0**
	1978	1,09 ± 0,17	2,64 ± 0,22	28,6**	1,75 ± 0,24	3,21 ± 0,32	12,8**	2,03 ± 0,25	3,77 ± 0,32	17,8**
Minor defects	1975	6,33 ± 0,26	6,31 ± 0,28	0,00	7,01 ± 0,20	6,59 ± 0,26	1,57	6,86 ± 0,24	6,56 ± 0,32	0,55
	1976	4,81 ± 0,22	5,32 ± 0,24	2,29	6,93 ± 0,35	6,65 ± 0,29	0,36	6,88 ± 0,35	7,12 ± 0,35	0,23
	1977	3,93 ± 0,19	3,61 ± 0,23	1,08	5,07 ± 0,19	4,53 ± 0,25	2,79	5,28 ± 0,26	5,35 ± 0,25	0,03
	1978	4,84 ± 0,23	5,22 ± 0,29	1,06	7,43 ± 0,40	8,04 ± 0,56	0,76	8,46 ± 0,48	9,31 ± 0,50	1,47

** = ($P < .01$)
(n.s.) = no significant
Q.T.T. = Quick Thermoresistance Test
S.T.T. = Slow Thermoresistance Test
C = phase-contrast microscopy
I = differential interference contrast microscopy
F = "F" test - Snedecor