Vol 15 No 1, p 85-87

VIABILITY ASSESSMENT OF CAMEL SPERMS USING HOECHST 33258 STAIN

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ABSTRACT

The proportion of living sperm in semen from 11 Jaisalmeri camels was assessed by means of a fluorescence staining technique using the Hoechst 33258 stain. The objectives were to study the head membrane integrity (%) of the spermatozoa and to make its comparison with the sperm motility (%) obtained with phase-contrast microscope. The head portions of dead spermatozoa get blue staining and give bright fluorescence under the microscope. The proportions of living and dead sperm in camel semen were readily identified through the use of Hoechst 33258. Mean sperm head membrane integrity (21.18±6.38%) was non-significantly higher in comparison to the mean motility (17.00±5.12%).

Key words: Camel, Hoechst 33258 stain, sperms, viability assessment

Advances in staining technology have provided new dimensions to study the functional capabilities of sperm. There are so many stains available, which can be used either alone, or in combinations to determine the sperm viability *viz.* rhodamine (R123), ethidium bromide (EtBr), carboxyfluorescein diacetate (CFDA), carboxydimethylfluorescein diacetate (CMFDA), propidium iodide (PI) and *Pisum sativum* agglutinin (PSA) etc. Each stain has its own property and stains different organelles, parts of the cells, spermatozoa, such as rhodamine for mitochondrial membrane potential and ethidium bromide to determine membrane integrity (Evenson *et al,* 1982). Sperm DNA and viability have been assessed with the bisbenzimide stains, Hoechst 33342 (Johnson *et al,* 1989) and 33258 (De Leeuw *et al,* 1991). These stains must be excited with ultraviolet (UV) light to emit blue fluorescence.

In a recent study, Hoechst 33258 dye has been used to judge the head membrane integrity of monkey spermatozoa (Li *et al,* 2005). The present study was sought to examine the staining characteristics of living and dead sperm from Jaisalmeri camels using Hoechst 33258 with fluorescence microscopy and to compare these results with motility percent.

Materials and Methods

Fluorescence microscopy for sperm head membrane integrity

Prior to use, Hoechst 33258 staining dye was dissolved in phosphate buffer saline (NaCl 8 gm; KCl 0.2 gm; Na_2HPO_4 1.15 gm; KH_2PO_4 0.2 gm in one litre double distilled water; pH 7.1) at the concentration of 0.5 mg/ml. 1 µl of freshly prepared 0.5 mg/ml Hoechst 33258 dye was added to 500 µl of semen sample and allowed to stain in dark at 37°C for 10 minutes. Spermatozoa were centrifuged at 250x g for 10 minutes to remove the excess stain. Sperm pellet was re-suspended in 50-500 µl prewarmed phosphate buffer saline. 10-20 µl of suspension was kept on a slide, covered with a cover slip and examined under fluorescence microscope. Sperm head membrane integrity was measured by counting non-damaged spermatozoa, defined as non-stained by Hoechst 33258 (Cross *et al,* 1986). The excitation and emission max for this dye are 346 and 460 nm, respectively. Under ultraviolet (UV) light, spermatozoa with intact membrane showed little or no blue fluorescence head, while spermatozoa with damaged membrane showed bright blue fluorescence. At least 150 spermatozoa were counted from different fields to assess the sperm head membrane integrity. The images were recorded under 60X magnification with Olympus CX41 microscope.

Phase contrast microscopy for motility examination

A small drop of semen kept on a pre-warmed slide with a cover slip on it. The slide was observed on a phase contrast Nikon microscope (200X magnification), at 37° to 40°C. The percentage of motile sperms with respect to non-motile sperms was observed in different fields.

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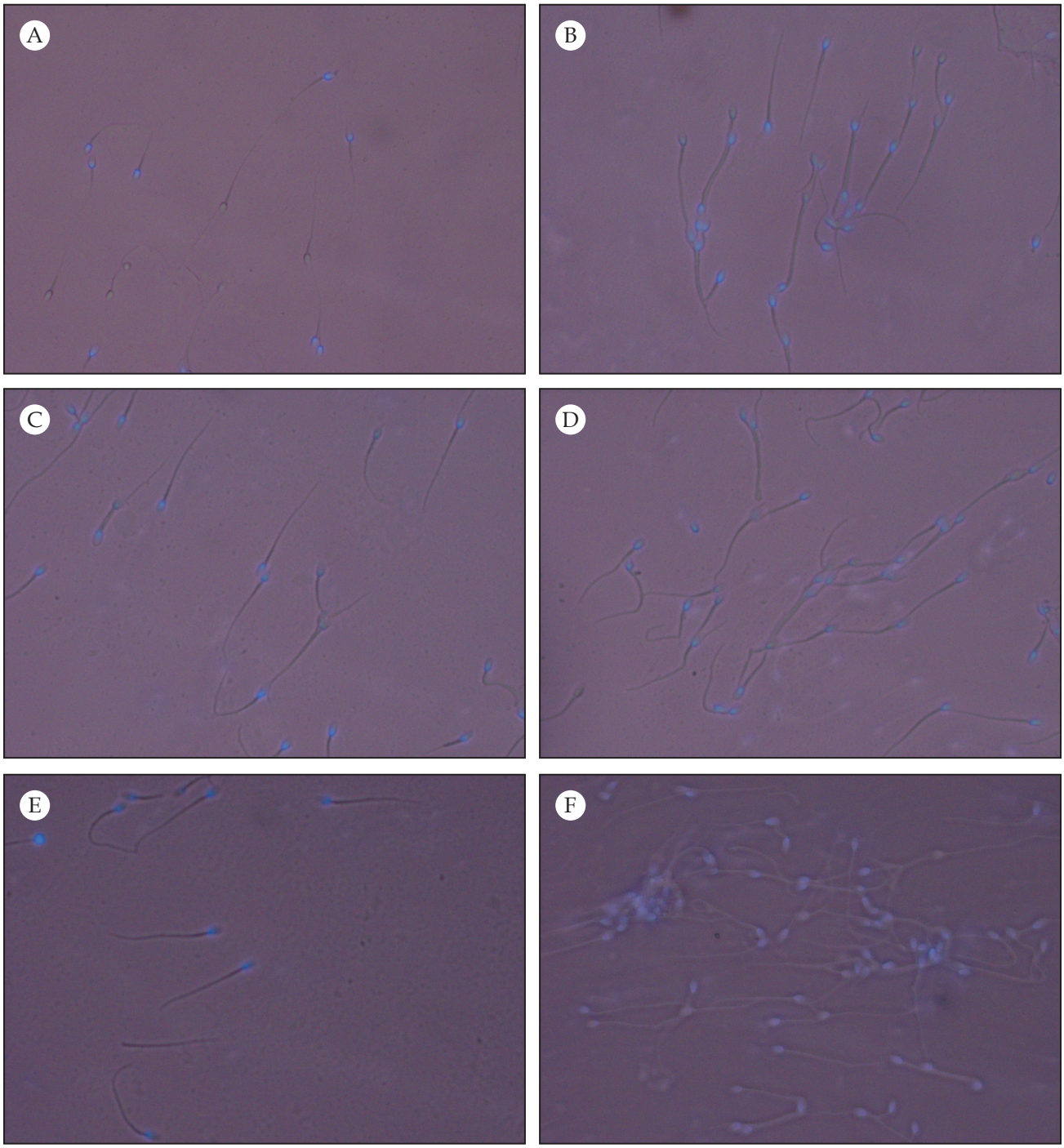

Fig 1. Camel spermatozoa stained with Hoechst 33258 and images were recorded under 60X magnification with Olympus CX41 fluorescence microscope. Clear view of live (unfluoresced) and dead (fluoresced) spermatozoa (A), abnormal spermatozoa with fused head and tails (B, C and D), dead spermatozoa without head portion (E) and clumping of the spermatozoa (F).

Results and Discussion

The integrity of the sperm head membrane was examined with fluorescent microscope after staining with Hoechst 33258 dye. This technique had an added advantage being its sensitivity and easy detection of dead spermatozoa. The head of dead (apparently non-motile) sperms exhibited bright blue staining under the fluorescence microscope. The live sperms did not give blue staining, but light yellow colours in the head portion were observed. Different types of observations were recorded from the semen samples of individual camel.

Fig 1A showed a clear view of live and dead spermatozoa. The live spermatozoa does not get

stained with Hoechst dye because they have intact cell membrane where as, dead spermatozoa appears bright blue in colour i.e. they get stained with Hoechst dye as they do not have intact cell membrane. Figs 1B, 1C, 1D showed abnormality found in the sperms. Abnormal sperms can be classified into different categories *viz*, double heads (Fig 1D) and absent heads (Fig 1E). However, as represented in Fig 1F and in most of the semen samples observed under fluorescence microscope; spermatozoa were found to be in the form of clumps.

The head membrane integrity (%) of the sperms was compared to the motility (%) and observations are given in table 1. The overall head membrane integrity (%) was found to be non-significantly higher compared to motility (%). From this study, it was revealed that Hoechst 33258 staining gave a better picture of the sperms and also estimates about live and dead sperms. However, this study should be carried out to a large number of semen samples to establish the facts.

As mentioned earlier, Hoechst 33258 stains must be excited with UV light to emit blue fluorescence. However, UV-generated fluorescence may be detrimental to cellular function and DNA integrity in some cell types. Therefore, stains having excitation in visible light should be preferred. Dual staining can be more useful in estimating the proportions of living, slightly damaged or dead sperms in semen samples (Gamer *et al*, 1994). In future, Flow cytometry could be more useful to detect differences in the staining proportions of sperm among the individual camels.

Table 1. Comparison of sperm head membrane integrity (%) and motility (%).

Sl. No.	Camel Number	Head membrane integrity (%)	Motility (%)
1	J-110	10	6
2	J-112	30	23
3	J-122	35	29
4	J-126	12	10
5	J-128	40	35
6	J-214	20	17
7	J-218	25	20
8	J-222	7	5
9	J-224	4	1
10	J-226	45	38
11	J-228	5	3
Mean±SE		21.18±6.38	17.00±5.12

Summary

Live and dead sperm populations can be identified after staining with Hoechst 33258 in semen from camels. The head of the dead sperm stained blue and light yellowish/bluish of the live sperms. It is observed from the study that correlation might be existing between morphologically normal sperm and sperm motility (%).

Acknowledgement

The authors are thankful to the Director, Central Institute of Arid Horticulture, Bikaner for extending the fluorescence microscope facility.

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