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Research Article

Acrosomal integrity of buffalo spermatozoa and its relationship to fertility

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ABSTRACT

Acrosomal integrity of neat semen of ten Murrah buffalo bulls was evaluated by giemsa staining. Eight bulls had >90 per cent spermatozoa with intact acrosomes, while in two bulls the intact acrosomes averaged 67.9 and 79.8 per cent. In these two bulls many ejaculates showed a high incidence of detached acrosome; mean values being 16.9 and 18.8 per cent as against 2.4 to 7.4 per cent in the remaining bulls. Two consecutive weekly ejaculates from a bull with a very low and high incidence of detached acrosome were frozen and used in field AI trial. The conception rate declined by 11.2 per cent when incidence of detached acrosome increased from 3.4 to 26.0 per cent. These results indicated that among various measurements of ejaculate quality, acrosomal evaluation would help to identify bulls with potentially lowered fertility.

Key words : Acrosomal integrity, fertility, buffalo, spermatozoa, semen evaluation

Anomalies of acrosomal cap are associated with infertility or sterility in bulls (Hancock, 1949; Rollinson and Mackinson, 1949; Saacke *et al.*, 1968). Some acrosomal abnormalities are hereditary (Hancock, 1949, 1953), while others may occur due to an adverse environmental effect (Blom, 1946). Perusal of the literature revealed scant information on the acrosomal integrity of buffalo spermatozoa. Therefore, this study was designed to evaluate the acrosomal integrity of buffalo spermatozoa and to ascertain its relationship to fertility.

MATERIALS AND METHODS

Semen from ten Murrah buffalo bulls was collected once weekly for ten weeks in artificial vagina. Acrosomal morphology was evaluated according to Watson (1975) with minor modifications (Pant, 2000). Two consecutive weekly ejaculates from a bull with very low and high incidence of detached acrosome, respectively, were frozen to evaluate the effect of detached acrosome on fertility. Oestrus was detected by owners based on behavioural signs and mucus discharge from the vulva. Animals were inseminated twice as per am : pm schedule. Pregnancy diagnosis was done per rectum between 45 to 60 days after breeding. The data for per cent normal (intact) acrosome were subjected to arcsin transformation before analysis of variance to assess differences between bulls and between ejaculates, while data on conception rate was compared by Chi-square test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

The results listed in Table 1 indicate that out of the 10 buffalo bulls, 8 gave consistently satisfactory semen as the incidence of intact acrosomes in the neat semen averaged ≥90 per cent. However, in the two bulls (# 5 and # 8) the acrosomal integrity showed considerable weekly variation and for ten consecutive weekly collections, the intact acrosome averaged 67.9 and 79.8 per cent, respectively. Analysis of variance of the data revealed significant differences between ejaculates ($P<0.05$) and between bulls ($P<0.01$). The decline in the percentage of intact acrosomes in the two bulls was associated with an increase in abnormal acrosomes, especially in the incidence of lost or detached acrosome or lost *galea capitis*. Based on ten consecutive weekly collections the incidence of detached acrosome averaged 18.8 and 16.9 percent in the two bulls, respectively. In the other eight bulls the incidence of detached acrosome averaged between 2.4 and 7.4 per cent. These detached acrosomes appeared as halfmoon-shaped structures floating free in the ejaculate (Pant, 2000).

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Table 1. Incidence (per cent) of intact and detached acrosomes in the neat semen of buffalo bulls (Mean±S.E.M.)

Bull No.	Intact acrosome	Detached acrosome
1	88.9±1.88	4.4±1.20
2	91.8±1.51	2.4±0.45
3	89.0±2.15	7.4±2.20
4	91.2±2.20	2.9±0.51
5	67.9±5.09	18.8±5.20
6	88.9±2.22	4.5±1.30
7	91.9±1.79	4.0±1.20
8	79.8±6.30	16.9±5.90
9	93.1±1.65	2.8±0.70
10	91.0±1.75	5.2±0.96

n = 10

This phenomenon of detachment of acrosomes observed in buffalo bull semen was first reported by Blom (1946) in bull and stallion semen. In our study, careful handling of semen (avoiding thermal and/or hypo-osmotic shock) ruled out the improper handling of semen as a cause of detachment of acrosome (Hancock, 1952; Blom, 1964). Moreover, we could not induce a high incidence of this condition by giving hypo-osmotic shock (1:1 dilution with 1.3% sodium citrate, osmolarity 100 mOsm) and cold shock (incubating neat semen for 10 min. in crushed ice) to neat semen. Furthermore, examination of wet-preparation under phase-contrast microscope also revealed the presence of free-floating halfmoon-shaped *galea capitis*, indicating that the high incidence of this condition observed in two bulls was indeed a natural phenomenon. Since the semen was being collected weekly, the high incidence of detached acrosomes in two bulls may not be attributed to epididymal aging (Hancock, 1952; Blom, 1964). Nevertheless, it would be of interest to see if a partial exhaustion test in a bull with a high incidence of detached acrosomes on a particular collection date would reduce the incidence of this condition.

Characteristics of semen from a Murrah bull on two separate collection days are shown in Table 2. It is apparent that in the second consecutive weekly collection, with the exception of per cent intact acrosome, all other characteristics of semen quality were within acceptable limits. The semen of second consecutive week had nearly 50 percent reduction in intact acrosomes associated with 26 and 28 per cent incidence of detached and ruffled acrosome, respectively. Pregnancy rates in buffaloes bred by AI to control semen (ejaculates of week 1) and to semen of consecutive weeks (week 2) are listed in Table 3. Perusal of the table reveals that the conception rate declined by 11.2 per cent following AI with semen having 26 per cent incidence of detached acrosome. Although, this reduction in fertility was not significant due to low population size, however, it is apparent that in biological terms fertility was compromised with the semen having high percentage of detached acrosome. We are not aware of similar study in the buffalo bulls. However, others have shown a high correlation between the percentage of intact acrosomes and fertility of frozen bovine spermatozoa after 2 and 4 hour of post-thaw incubation (Sacke and White, 1972) and evaluation of acrosomal integrity following incubation has been helpful in monitoring the quality of bovine spermatozoa (Saacke and Marshall, 1968, Saacke, 1970).

In conclusion, these results indicate that acrosomal evaluation, among ejaculate quality measurements, would help to identify bulls with potentially lowered fertility. Additionally, the apparent sensitivity of the acrosome to some subtle environmental / physiological changes indicates more regulated approaches to management of such problem bulls for maximum availability of useful spermatozoa. These managemental approaches may include increased frequency of

Table 2. Characteristics of neat and frozen-thawed semen from a Murrah bull on two separate collection days

	Seminal Characteristics	Week 1	Week 2
	Volume (ml)	6.3	4.0
	Sperm Conc. (x 10^6/ml)	1218.5	1567.0
	Sperm conc./ejac. (x10^6)	8896.3	6261.5
	Live sperm (%)	85.0	72.0
Neat semen	Intact acrosome (%)	90.1	46.0
	Detached acrosome (%)	3.4	26.0
	Ruffled acrosome (%)	5.9	28.0
	Knobbed acrosome (%)	0.5	–
	Progressive motility (%)	70.0	65.0
Frozen-thawed semen	Progressive motility (%)	60.0	55.0

Table 3. Pregnancy rates in buffaloes bred by AI to control semen (ejaculates of week 1) and ejaculates of next week (week 2) with 26 per cent spermatozoa devoid of *galea capitis*

Ejaculate	No. inseminated	No. examined for pregnancy (%)	No pregnant (%)
Week 1	57	56 (98.2)	27 (48.2)
Week 2*	54	54 (100.0)	20 (37.0)

*Detached acrosome 26.0 per cent

collection and a change in the mineral status of the feed supplement. The latter comment is based on a recent study in which high level of zinc in the diet was associated with a high incidence of detached acrosome in the bovine (Hansel, W., Personal communication). Further studies are needed to test these suggestions.

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