ASSESSMENT OF MOTILITY AND VELOCITY PARAMETERS OF FRESHLY EJACULATED SPERMATOZOA OF *BUBALUS BUBALIS* AND *BOS INDICUS* BULLS THROUGH COMPUTER ASSISTED SEMEN ANALYZER

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ABSTRACT

Fresh semen ejaculates (90) of 15 mature bulls, 5 each of Jafarabadi, Mehsana and Crossbred (HF x Kankrej) breeds were studied for sperm motility and velocity parameters through computer assisted semen analyzer (CASA). The mean values of motile and progressively motile sperm in the semen of Jafarabadi and Mehsana buffalo bulls were significantly ($P<0.05$) higher than in crossbred bulls. The average path velocity of buffalo sperms were significantly higher than those in cow bulls. Moreover, semen of Jafarabadi bulls was better than Mehsana buffalo bulls in these regards. Among the mean values of other velocity parameters, viz., amplitude of lateral head displacement (ALH, μm), beat-cross frequency (BCF, Hz), straightness (%), linearity (%), elongation (%) and sperm area (μm2), none differed significantly between Jafarabadi, Mehsana and HF x K crossbred bulls, except BCF, which was significantly ($P<0.01$) higher for buffalo than in crossbred bulls. The velocity distribution of sperms in terms of rapid, medium, slow motile and static did not differ between Jafarabadi and Mehsana bulls, but the values of rapidly motile sperm in crossbreds were significantly lower and medium motile and static sperm were higher than in buffaloes. Semen of crossbred bulls in general appeared relatively poor than Mehsana buffalo and the Jafarabadi sperms were better in terms of all the traits evaluated through CASA.

INTRODUCTION

Evaluation of semen of breeding bulls is of paramount importance (Garner, 1997). Although several methods are available to evaluate the quality of semen sample, subjective evaluation using optical microscopy is by far the most commonly used one (Christensen et al., 2005). Conventional semen parameters routinely examined are concentration, percentage of motile spermatozoa and their morphology (Vantman et al., 1988). The subjective evaluation of semen has been shown to be relatively inaccurate, imprecise (Christensen et al., 2005), time consuming, and the subjective motility judgment largely depends on the level of training and skills of the investigator (Knuth et al., 1989). Computer Assisted Semen Analyzers (CASA), therefore, have been introduced mainly to save time in the evaluation procedure, to avoid errors due to subjective evaluation of different technicians, to improve the accuracy of data collection, and to gather population values for aspects of sperm kinematics (Johnson et al., 1996). The kinematic values determined for each spermatozoa cover the velocity of movement, viz., curvilinear velocity (VCL), straight line velocity (VSL) and average path velocity (VAP), the width of the sperm head's trajectory and frequency of the change in direction of the sperm head (David et al., 1981; Mortimer et al., 1990) and thus provide qualitative assessment of sperms. The present study was therefore aimed to qualitatively compare the kinematics of Jafarabadi, Mehsana and HF x Kankrej crossbred bulls' spermatozoa using CASA.

MATERIALS AND METHODS

The study was carried out at the State Frozen Semen Production and Training Institute, Patan, Gujarat during March to April 2011. Fifteen sexually mature healthy breeding bulls, 5 each of Jafarabadi, Mehsana and HF x Kankrej (F1) breeds, aged 3-8 years, were included in the study. All these bulls were in good health and under optimal veterinary care. They were maintained in nearly identical nutritional and managerial conditions throughout the period of study and were under regular twice a week semen collection programme. Immediately after collection, the semen samples were diluted 1:100 with 0.9% w/v normal saline (semen samples 40 µl + normal saline 3960 µl). 20 µl of mixed semen sample was put by a pipette into Leja slide (4 chambers) of Computer Assisted Semen Analyser (Hamilton Thorne Biosciences, IVOS Version 12.3, Beverly, MA) and the analysis set up appropriate for fresh extended semen was selected as per the manufacturer's instruction. In all, 90 semen ejaculates (6/bull) were studied for various standard sperm kinematics traits. The data were analyzed statistically using CRD and Duncan's NMRT to test the differences between breeds (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The CASA analysis of fresh semen (Table 1) revealed the overall mean motility parameters like total motile and progressively motile spermatozoa to be significantly (P<0.05) higher in semen of Jafarabadi and Mehsana buffalo bulls as compared to HF x K crossbred bulls. However, the values for Jafarabadi and Mehsana buffalo bulls were statistically at par for both the traits.

The mean values of average path velocity (VAP) and curvilinear velocity (VCL) for HF x K crossbred bulls spermatozoa were significantly (P<0.05) lower than in Jafarabadi and Mehsana buffalo bulls, which however did not differ in these regards. The mean values of straight line velocity (VSL) differed highly significantly (P<0.01) among the three breeds. The VSL value for Jafarabadi buffalo sperms being the highest followed by Mehsana buffalo and the lowest for HF x K crossbred bulls. Overall, Jafarabadi buffalo spermatozoa were significantly superior in terms of velocity parameters as compared to Mehsana buffalo and crossbred bulls (Table 1).

Amongst other sperm velocity parameters, which were assessed by CASA viz. the Amplitude of Lateral Head displacement (ALH, µm), Beat-Cross Frequency (BCF, Hz), Straightness (STR, %), Linearity (LIN, %), Elongation (ELG, %) and Area (ARE, µm2) of sperms, the mean values of none of them differed significantly between Jafarabadi, Mehsana and HF x K crossbred bulls, except BCF, which was significantly (P<0.01) higher for buffalo semen than in HF x K crossbred bulls sperms (Table 2). This could be due to gross difference in the size/dimensions of cattle and buffalo spermatozoa, the later being smaller in all dimensions.

The present findings on CASA analysis of some of the important semen traits of cow bulls and buffalo bulls compared favourably with the reports of Tardif et al. (1997), Ferrell et al. (1998), Karthikeya (2003), Rengarajan (2004) and Hoflack (2006) in bovines. Ramachandran et al. (2006) noted the mean values of MOT, VSL, VCL, VAP, LIN and ALH as 57.27 ± 3.20 %, 34.18 ± 1.26 µm/s, 96.40 ± 3.80 µm/s, 62.13 ± 2.28 µm/s, 44.97 ± 4.20 % and 10.04 ± 0.71 µm, respectively, in fresh semen of Sahiwal bulls. Hoflack (2006) compared overall results of CASA for fresh semen of HF and Belgian Blue (BB) bulls and noted significant differences in all the assessed parameters. The percentages of total and progressively motile spermatozoa and the semen velocity parameters were significantly (P<0.01) lower in the Belgian Blue breed. The ALH was higher combined with a lower BCF for the Belgian Blue spermatozoa. Mandal et al. (2003) observed significantly lower MOT, VSL, VCL and VAP in buffalo ejaculates with <50 % HOS-reactive spermatozoa as compared to those with >50 % HOS-positive spermatozoa.

In the present study, the mean values of rapidly motile, medium motile and static spermatozoa differed significantly between cow bulls and buffalo bulls, although the values in Jafarabadi and

Mehsana buffalo bulls were at par. Mean values of rapidly motile sperm were significantly (P<0.01) higher in Mehsana and Jafarabadi buffalo bulls, whereas the values of medium motile and static sperms were significantly (P<0.01) higher in HF x K crossbred bulls (Table 3).

Table 1: Mean (±SE) motility and velocity parameters of fresh spermatozoa of Jafarabadi, Mehsana and HF x K crossbred bulls

Breed	Motile Sperm %	Progressive Motile %	Av. Path Velocity (VAP, µm/s)	Straight-line Velocity (VSL, µm/s)	Curvilinear Velocity (VCL, µm/s)
Jafarabadi	79.77±1.62[a]	61.80±1.85[a]	114.15±2.28[a]	99.97±2.09[a]	181.30 ±4.19[a]
Mehsana	78.90±1.22[ab]	61.37±1.58[a]	108.75±2.59[a]	93.63±2.14[b]	176.72±6.12[a]
HF x K (F1)	74.73±1.71[b]	51.57±2.61[b]	100.37±2.61[b]	86.25±2.15[c]	158.93±6.46[b]

Means bearing common superscript within the column do not differ significantly (P>0.05).

Table 2: Mean (±SE) velocity parameters of fresh spermatozoa of Jafarabadi, Mehsana and HF x K crossbred bulls

Breed	ALH(µm)	BCF (Hz)	STR (%)	LIN (%)	ELG (%)	ARE(µm^2)
Jafarabadi	6.57±0.17	38.51±0.75[a]	87.63±0.84	60.73±1.47	43.07±1.99	20.23±0.58
Mehsana	6.63±0.27	37.64±0.64[a]	86.43±1.04	60.00±2.58	46.83±2.41	22.13±0.99
HF x K (F1)	6.87±0.31	32.79±0.81[b]	86.63±1.13	61.93±2.24	44.43±1.51	22.13±0.81

Means bearing common superscript within the column do not differ significantly (P>0.05).

ALH= Amplitude of lateral head displacement, BCF= Beat cell frequency, STR= Straightness, LIN= Linearity, ELG= Elongation, ARE= Area

Table 3: Mean (±SE) velocity distribution (%) of fresh spermatozoa of Jafarabadi, Mehsana and HF x K crossbred bulls

Breed	Rapid motile	Medium motile	Slow motile	Static
Jafarabadi	71.13 ± 2.17[a]	8.86 ± 1.75[b]	14.33 ± 1.49	5.87 ± 0.58[b]
Mehsana	71.70 ± 1.62[a]	7.87 ± 1.32[b]	12.53 ± 1.20	8.53 ± 0.77[b]
HF x K (F1)	59.60 ± 2.50[b]	15.03 ± 1.88[a]	16.17 ± 1.39	9.17 ± 0.63[a]

Means bearing common superscript within the column do not differ significantly (P>0.05).

The literature on velocity distribution of motile sperm of cattle and buffalo bulls is meager. Hoflack (2006) recorded fewer rapid but more slow and static spermatozoa in Belgian Blue (BB) bulls, while a similar proportion of medium velocity sperm occurred in both BB and HF breeds. Kumar et al.

(2010) observed significant (p<0.05) effect of breed of rams on sperm motion characteristics including rapid, medium, and slow motile sperms.

The findings in general indicated that the sperm motion characteristics, viz., motile, progressive motile sperm, VAP, VSL, VCL, ALH, BCF, straightness, linearity, elongation, sperm area were significantly inferior in fresh semen of HF x Kankrej (F1) bulls as compared to Mehsana and Jafarabadi buffalo bulls, and that the CASA provided the most accurate, quick and qualitative assessment of sperm kinematics of fresh semen of both cow bulls and buffalo bulls. Although, how far these traits are correlated with fertilizing potential of sperms needs further study.

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