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Short Communication

In vitro assessment of seminal traits and fertility parameters in fresh semen of Sahiwal bulls

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ABSTRACT

Eight Sahiwal bulls maintained at Artificial Breeding Complex, National Dairy Research Institute, Karnal, Haryana were used to evaluate the seminal attributes and fertility parameters from fresh semen samples. The overall least squares means for various seminal traits and fertility parameters were reported. The effect of bull was significant (P<0.01%) on parameters like volume, progressive motility, per cent motility and on velocity of average path (P<0.05). The positive and the significant (P<0.05) correlations amongst the seminal traits and their significant (P<0.05) association with the fertility parameters suggest that consideration of the fertility traits along with the seminal traits would be valuable for selecting bulls to be used for artificial insemination programme.

Key words: Semen evaluation, *in vitro* fertility tests, CASA, Sahiwal bull.

Evaluation of breeding bull for fertilizing ability is essential for any breed improvement program. The periodical evaluation of semen quality of bulls becomes inevitable due to their extensive use through artificial insemination and helps in early detection of impaired fertility. However, standard analysis procedures like volume, per cent motility, live sperms etc in most of the semen processing laboratories are limited in their use for predicting the sperm fertilizing ability. These parameters in semen evaluation are highly subjective and do not correlate well with actual fertility of bull (Jasko *et al.*, 1988). The functional sperm tests like acrosomal integrity, hypo-osmotic swelling test and computer assisted semen analysis (CASA) parameters are objective and highly associated with fertility of bulls (Correa and Zavos, 1994; Revell and Mrode, 1994). Limited information is available on seminal traits with fertility parameters of indigenous bulls. The present study was, therefore, carried out to evaluate the fresh semen of Sahiwal bulls both subjectively as well as objectively for its subsequent use as liquid / frozen semen in dairy cattle improvement program.

Eight Sahiwal bulls maintained at Artificial Breeding Complex, National Dairy Research Institute, Karnal India were used for present study. These bulls were of similar body size, weight and age (average 450 kg; 4-5 years old) and were kept individually in loose housing system under uniform management conditions. Semen from each bull was collected twice per week at an interval of 30 minutes by using a teaser bull. Physical evaluation of semen was done immediately followed by microscopic evaluation, after diluting the neat semen in 1:10 ratio with Tris diluent. The physical appearance, volume, mass activity, progressive motility and sperm concentration of neat semen were estimated as per standard procedure. The estimation of non-eosinophilic sperms and total abnormal sperms were done as per Bloom (1950) and Hancock (1951). A Computer Assisted Semen Analyser (CASA) (" Cell track/S", VP 100 Motion Analysis Corporation, Santa Rosa, C. A.) was used to determine various motility parameters like per cent motility [MOT (%)], straight-line velocity [VSL (μ/sec)], curvilinear velocity [VCL (μ/sec)], mean linearity [LIN (%)], lateral head displacement [ALH (μ)]

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d velocity of average path [VAP (μ/sec)]

A thin smear was prepared, air dried and kept in % formaldehyde solution for 30 minutes at room mperature for acrosomal integrity test. Then slides ere washed in double distilled water, air dried and kept staining jar having thoroughly mixed solution of 3 ml f giemsa stock solution and 2 ml phosphate buffer lution in 45 ml of double distilled water for 20-24 hrs 37°C in the incubator. The stained slides were washed, ir dried and utilized for counting sperms having intact crosomes. HOS reacted sperms were counted as per yendran *et al.* (1984). The least squares one-way odel was used to analyse the non-orthogonal perimental data after transforming the percent values y arcsine transformation. The relationship between eminal attributes and fertility parameters were obtained y simple correlation coefficients (Snedecor and ochran, 1994).

The least squares means for seminal attributes d CASA parameters are depicted in Table 1. The verall volume was 3.36 ± 0.14 ml (range from 2.10±0.35 l to 4.60±0.37 ml) and it varied significantly (P<0.01) mongst bulls. Differences in volume is due to variation the androgen dependent accessory glands particularly estosterone concentration (Tomar and Kanaujia, 1970). The physical appearances of semen samples were thick creamy (19.23%), thin creamy (12.82%), milky (21.79%), watery (8.97%), lemon (11.53%) and thick lemon (26.54 %) from 78 ejaculates. The present results showed that 90% semen samples can be utilised for further processing. Results are in agreement with those reported by Kumar (1993).

The overall least squares means for mass activity was 2.57±0.08 which did not differ significantly among bulls. The present result was in close agreement with earlier reports (Panwar, 1989; Kumar, 1993; Keshava, 1996) in Sahiwal bulls. The overall mean progressive motility was 59.99 % (range 54 to 66%) and significantly (P<0.05) differed among bulls. The overall mean for sperm concentration (10^6/ml), non eosinophilic sperm count (%) and the total abnormal sperm count (%) were 1505.26±172.20, 63.35±1.13 and 6.72±0.64, respectively. The mean seminal attributes recorded in present study were of good quality and meet out the standards set for semen freezing.

The overall means for CASA parameters are given in table 1. The MOT (P<0.01) and VAP (P<0.05) differed significantly among bulls. Computer assisted semen analyser measured the motile sperms in the present study ranging from 41-69.5% Vs 54-65% motility observed in subjective evaluation. The CASA provided highly repeatable estimates of sperm motility, which was in consonance with the values reported by Farrell *et al.* (1998).

The overall means for HOS reacted and acrosome intact sperms were 60.96 ± 1.50 and 71.31±0.77 %, respectively (Figure 1). The motile sperm though reach the site of fertilisation, sperm with damaged acrosome fail to fertilize resulting in conception failure. The mean HOS reacted and acrosome intact sperms in the present study were higher than the values reported by Keshava (1996) in Sahiwal bull semen. Many reports have clearly indicated that the CASA parameters like MOT, VSL, VCL, LIN, ALH, VAP (Aitken, 1990; Kjaestaed *et al.*, 1993, Jadhav, 1998), acrosome intact sperms (Lenz et al, 1988; Kumar 1992) and HOS reacted sperms (Breederman and Foote, 1969) had significant correlation with non- return rate in bulls.

The mass activity and progressive motility had positive significant correlation (P<0.01) with per cent

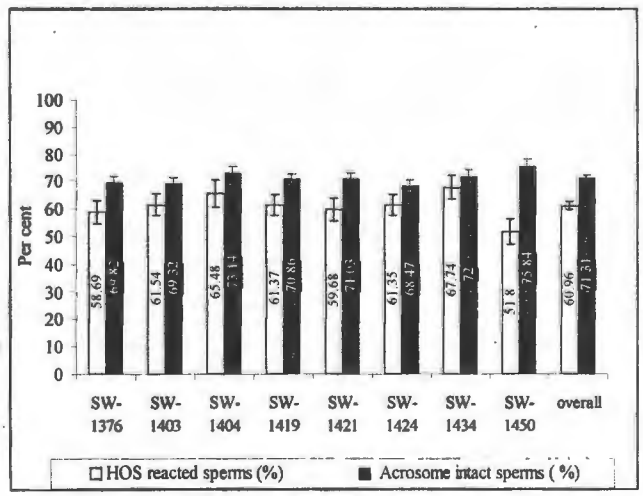

Fig 1. Least squares means for HOS reacted and acrosome intact sperms in fresh semen of Sahiwal bulls.

Table 1. Least squares means for seminal attributes and CASA parameters in fresh semen of Sahiwal bulls.

Traits \ Bull No	Volume (ml)	M A (0-5 scale)	PM (%)	S C (10^6/ml)	NESC (%)	TASC (%)	MOT (%)	VSL (μ/sec)	VCL (μ/sec)	LIN (%)	ALH (μ)	VAP (μ/sec)
1 (10)	4.6 ± 0.37	2.65 ± 0.25	59.99 ± 2.80	1304.39 ± 72.84	65.79 ± 3.10	9.21 ± 1.77	60.9 ± 3.30	29.37 ± 3.46	82.3 ± 10.43	63.98 ± 11.54	10.78 ± 1.95	61.74 ± 6.26
2 (11)	2.1 ± 0.35	2.68 ± 0.23	55.45 ± 2.68	1682.88 ± 450.83	65.08 ± 2.96	10.41 ± 1.69	59.63 ± 3.15	34.15 ± 3.30	96.96 ± 9.95	39.2 ± 11.00	10.12 ± 1.86	54.8 ± 5.97
3 (7)	3.14 ± 0.44	2.92 ± 0.29	66.42 ± 3.36	1433.36 ±565.16	68 ± 3.71	5.74 ± 2.11	49.85 ± 3.95	29.71 ± 4.14	87.81 ± 12.47	48.75 ± 13.79	10.78 ± 2.33	52.18 ± 7.48
4 (13)	3.23 ± 0.33	2.15 ± 0.22	58.31 ± 2.46	1619.85 ± 414.71	60.32 ± 2.72	5.32 ± 1.55	51.23 ± 2.89	38 ± 3.03	98.5 ± 9.15	41.23 ± 10.12	7.53 ± 1.71	72.43 ± 5.49
5 (9)	3.22 ± 0.39	2.56 ± 0.26	62.78 ± 2.96	1226.08 ± 498.42	59.83 ± 3.27	5.11 ± 1.86	62.33 ± 3.48	37.22 ± 3.65	115.78 ± 11.00	38.06 ± 12.16	10.06 ± 2.06	68 ± 6.60
6 (11)	4.4 ± 0.35	2.27 ± 0.23	57.54 ± 2.68	1473.32 ± 450.84	62.05 ± 2.96	6.57 ± 1.68	41.09 ± 3.15	35.93 ± 3.30	93.59 ± 9.95	44.44 ± 11.00	14.16 ± 1.86	47.68 ± 5.97
7 (9)	2.98 ± 0.39	2.33 ± 0.26	54.44 ± 2.96	1551.59 ± 498.42	55.93 ± 3.27	5.73 ± 1.86	63.66 ± 3.48	35.55 ± 3.65	97.21 ± 11.00	43.53 ± 12.16	10.17 ± 2.06	67.45 ± 6.60
8 (8)	3.23 ± 0.42	3 ± 0.28	65 ± 3.14	1750.62 ± 528.65	69.73 ± 3.47	5.63 ± 1.98	69.5 ± 3.69	33.49 ± 3.87	99.06 ± 11.67	40.53 ± 1.90	6.73 ± 2.18	72.72 ± 7.00
Overall (78)	3.36** ± 0.14	2.57 ± 0.08	59.99* ± 1.02	1505.26 ± 172.20	63.35 ± 1.13	6.72 ± 0.64	57.27** ± 3.20	34.18 ± 1.26	96.40* ± 3.80	44.97 ± 4.20	10.04 ± 0.71	62.13 * ± 2.28

Values in parenthesis are number of observations. MA- Mass activity, PM - Progressive motility (%), SC- Sperm concentration, NES - Noneosinophilic sperm count, TASC- Total abnormal sperm count. ** Significant ($P<0.01$), * Significant ($P<0.05$).

eosinophilic sperms (r = 0.47, 0.48), acrosome intact erms (r = 0.39, 0.57) and MOT (r = 0.29, 0.58) in esent study. Similarly, acrosome intact sperms had sitive and significant correlation with MOT (r=0.41; <0.01), VCL (r=0.23; P<0.05) and noneosinophilic erms (r=0.25; P<0.05). However, HOS reacted erms had negative and significant correlation with LIN r=-0.39; P<0.01). Present results are in agreement ith those reported by Pathak *et al.* (1990), Sharma *et l.* (1990) and Nagy *et al.* (2000).

The per cent progressive motility, non-eosinophilic erms, acrosomal integrity and MOT are highly rrelated among each other, therefore, these seminal rameters should be given due importance to get higher nception rate while selecting the bulls for artificial semination program.

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