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Evaluation of seminal parameters associated with subfertility in sahiwal bulls

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Abstract
The present study was conducted on Sahiwal bulls maintained at Artificial Breeding Research Centre (ABRC), National Dairy research Institute (NDRI), Karnal, with an objective to evaluate seminal parameters associated with sub-fertility. Based on the available record at ABRC, 9 sahiwal bulls were selected and classified into three categories, viz, 3 bulls in poor semen freezability, 3 bulls in poor semen quality and 3 bulls in normal. All the seminal parameter evaluated under study i.e., mass motility, individual motility, live percent (non-eosinophilic) count, sperm concentration, hypo-osmotic swelling test, and post-thaw motility showed significant association between different categories of bull ($P<0.05$) except ejaculate volume and A.I %. The normal bulls revealed comparatively higher mean values for all the seminal parameters under study that need to be exploited for routine seminal examination for selection and propagation of superior germplasm. However, validation of results on large number of samples needs to be warranted.

Keywords: Sahiwal, semen, acrosomal integrity, spermatozoa

Introduction
The success of reproduction of bulls is the product of many facets, namely male or female factor, accurate detection of estrous and the efficiency of the artificial insemination workers. Among these the male factor is of prime importance as the dissemination of superior male germplasm is faster with the adoption of artificial insemination along with associated aspects of semen freezing and dissemination to distant locations even in different countries.

There is a significant demand of elite Sahiwal bullsemen throughout the country due to its superior lactating ability, adaptability in harsh environment, disease resistance and sustainability on poor feed and fodder resources.The prospective males to be bulls are usually selected and reared up to the age of 2-3 years prior to their induction into progeny testing programme. Hence there is always a need of early evaluation of fertilizing ability of male. The procreation potential of male should be accurately assessed to augment reproduction performance, which is accompanied by enhanced production efficiency [2].

Subfertility and fertility are very important issues for appropriate management of reproduction. Subfertility results in reduced fertility with prolonged time of unwanted non-conception. In case of subfertility occurrence of less than optimal sperm quality including sperm morphology, motility and count, poor libido, non-freezability and anomalies in semen reduce the chance of conception without complete prevention or spontaneous occurrence and are responsible for disposal of breeding bulls. Fertility refers to the actual ability of an individual to produce offspring [17].

Mukhopadhyay (2008) [11] carried out an extensive study on subfertility problems and significantly important reason leading to culling of breeding males. Subfertility problems were found to be the significantly important reason leading to culling of breeding males. During the periodof15 years, 29.41% of the reserved Sahiwal males, were disposed due to various subfertility problems. Compilation of the disposal pattern of Sahiwal bulls at NDRI herd revealed that poor libido accounts for culling of 22.55% of the reserved males of Sahiwal. Sahiwal bulls produce 51.96% freezeablesemen. The present study was undertaken with an objective to identify marker related to seminal parameters associated with subfertility in Sahiwal bulls.

Materials and Methods

Experimental design

The present investigation was conducted on Sahiwalbullsmaintained at Artificial Breeding Research Centre of NDRI, Karnal Haryana. The study was carried out on both problematic and normal bulls, primarily concentrating on semen evaluation techniques. Bulls for research purpose will be selected on the basis of poor semen freezability (<35%), poor semen quality (Initial motility <40%) and normal bulls (regular donor of semen Post thaw motility >35%, Initial motility >40%) as per their record maintained in ABRC, NDRI, Karnal, India. The investigation was carried out on nine Sahiwal bulls. These nine animals were classified into 3-categories, *viz.*, 3 bulls in poor semen freezability, 3 bulls in poor semen quality and 3 bulls in normal.

Semen collection

Two successive ejaculates were collected from the bulls using sterilized bovine artificial vagina (IMV model-005417) maintained between 42-45°C, over a male dummy bull. Soon after collection, each ejaculate was placed in a water bath at 30°C and various standard laboratory tests for semen volume, mass motility (MM), individual progressive motility (IPM), non-eosinophilic spermatozoa count (NESC), sperm concentration (SCON) (million/ml) [15], hypo-osmotic swelling test (HOST) [5], acrosomal integrity (AI) andpost thaw motility was assessed on 20X objective using a phase-contrast microscope equipped with 37^0C heating stage.

Statistical analysis

The degree of association among various seminal attributes was estimated by Pearson's correlation coefficient method and significance was tested by Student's *t-test*.SPSS 11 statistical software was used for data analysis. Statistical analysis was performed after arcsine transforming the percentage values. Statistical significance was set at 0.05 probability level. If the effect was found significant, comparison of means was done by Duncan Multiple Range Test. Results are expressed as Mean ± Standard Error of Mean.

Results and discussion

The semen quality parameters play a significant role in order to estimate the quality and fertility of bulls. The results of least squares mean and correlation coefficients among different semen quality parameters are presented in Tables 1-2.

The overall least squares mean of semen ejaculation volume was 4.70 ± 0.28 ml (Table 1). Similar values were reported by [8, 4]. Lower values were reported by [6, 12, 16, 13, 10]. The semen ejaculation volume was not significantly different between different categories of bulls. However, volume of semen was comparatively more in normal bulls.

The average MM in the present study was 2.57 ± 0.10 (Table 1). The results are in consonance with the findings reported by [12, 13, 4], however, lower values were reported by [8], and [16]. Higher values compared to the present study were reported by [6, 10, 7]. MM was significantly inferior in poor semen quality category bulls than those of bulls belonging to poor freezability and normal categories (P<0.05).

The least squares mean of IPM was 67.04 ± 0.16% (Table 1). The obtained result is in agreement with the values reported by [3], whereas lower values were reported by [1]. Comparison within the categories showed that IPM% was significantly lower in poor semen quality animals than those of poor freezability and normal categories bulls (P<0.05).

The overall least squares mean values of NESC were 82.58 ± 0.18% (Table 1). Correlated mean values for NESC % of Sahiwal bull was reported by [3, 16]. However lower values were reported by [9]. The results further expressed that poor freezable and normal animals had significantly higher NESC than those of poor semen quality (P<0.05). After staining dead spermatozoa develop pink coloration, whereas live spermatozoa remain white in color.

The findings of the present study indicated that the SCON (million/ml) was significantly influenced by categories of bulls (P<0.05). SCON was found to be 840.34 ± 38.07 million /ml (Table 1). The results of the present study revealed that the SCON value of poor freezability and normal animals was higher significantly from poor semen quality category bulls.

Least squares mean value of HOST was 54.64 ± 0.23% (Table 1). Lodhi *et al.* (2008) [9] reported higher value. The HOST % estimates revealed that least squares mean of poor semen quality animals was significantly lesser than the poor freezability and normal animals (P<0.05). Tail piece of HOST reacted spermatozoa get curved and tail piece of HOST non-reacted spermatozoa remains straight.

Aggregated least squares mean of AI for Sahiwal in present study was observed as 97.05 ± 0.02% (Table 1). Corresponding AI estimates were reported by [18]. Value was higher in present study as compared with result reported by [9]. The least squares mean of the present investigation revealed that there were no significant differences in AI % between categories of bulls. AI plays a vital role in the semen quality.

Average of the post-thaw motility in present investigation was found to be 45.95 ± 0.01% (Table 1). Higher value than the value observed in our study was reported by [10]. Statistical analysis revealed that the post-thaw motility of poor freezability bulls was significantly lesser from post- thaw motility of normal bulls (P<0.05).

Correlation among various semen parameters in Sahiwal bulls

The correlation between sperm concentration with mass motility and the correlations of individual motility with mass motility and sperm concentration were positive and highly significant (p<0.01) (Table 2). Similarly, the association of HOST (%) with mass motility, sperm concentration, individual motility and live spermatozoa was positive and highly significant. The correlation between post-thaw motility with mass motility, sperm concentration, individual motility, live spermatozoa and HOST were highly significant. Whereas, the correlations between live spermatozoa (%) with mass motility, sperm concentration and individual motility were significant only. The correlation of acrosomal integrity with sperm concentration, live spermatozoa and HOST were significant (P<0.05). Overall, the results indicated positive and significant association amongst various semen characteristics except ejaculate volume in Sahiwal bulls.

Conclusion

Poor libido, poor semen quality and non-freezability of semen are some of the important etiologies of subfertility. These conditions are vital constraints in the production and propagation of superior germplasm of Sahiwal bulls. In present study, all the seminal parameters except ejaculate

volume and A.I % were highly significant (p<0.05) between categories of bulls. Normal Sahiwal bulls have comparatively higher mean values for all the seminal parameters under study. The information generated need to be validated on large sample size and a suitable range of each seminal parameter need to be fixed that can be exploited for regular screening and selection of superior bulls for genetic improvement of our indigenous cattle.

Table 1: Least square means ± S.E of various semen parameters under different bull categories

Parameter / Category	Poor freezability	Poor semen quality	Normal	Overall
Volume (ml)	4.03 ± 0.45	4.90 ± 0.55	5.16 ± 0.45	4.70 ± 0.28
MM	3.10^{b} ± 0.16	1.40^{a} ± 0.20	3.23^{b} ± 0.16	2.57 ± 0.10
IPM (%)	73.87^{b} ± 0.41	45.78^{a} ± 0.61	79.43^{b} ± 0.41	67.04 ± 0.16
NESC (%)	85.92^{b} ± 0.45	68.83^{a} ± 0.68	90.41^{b} ± 0.45	82.58 ± 0.18
SCON (millions/ml)	962.16^{b} ± 61.05	500.00^{a} ± 74.78	1058.86^{b} ± 61.05	840.34 ± 38.07
HOST (%)	65.22^{b} ± 0.59	29.73^{a} ± 0.89	68.86^{b} ± 0.59	54.64 ± 0.23
AI (%)	97.80 ± 0.05	96.91^{a}± 0.07	96.36 ± 0.05	97.05 ± 0.02
Post thaw motility (%)	33.85^{a} ±0.03		58.28^{b} ±0.03	45.95 ± 0.01

MM= Mass Motility, **IPM**= Individual Progressive Motility, **NESC**= Non-eosinophilic Spermatozoa Count, **SCON**= Sperm Concentration, **HOST**= Hypo-osmotic Swelling Test, **AI**= Acrosomal Integrity, (P<0.05)

Table 2: Correlation matrix among various semen parameters

	Volume	MM	SCON	IPM	NESC	HOST	AI	Post thaw motility
Volume	1							
MM	-0.161	1						
SCON	-0.05	0.9221**	1					
IPM	-0.257	0.9214**	0.8436**	1				
NESC	-0.315	0.8701**	0.8465**	0.893**	1			
HOST	-0.192	0.9518**	0.9158**	0.9379**	0.9**	1		
AI	-0.127	0.5573	0.6399*	0.5996	0.6207*	0.6315*	1	
Post thaw motility	0.0667	0.9003**	0.9229**	0.8584**	0.7909**	0.8689**	0.5037	1

MM= Mass Motility, **IPM**= Individual Progressive Motility, **NESC**= Non-eosinophilic Spermatozoa Count, **SCON**= Sperm Concentration, **HOST**= Hypo-osmotic Swelling Test, **AI**= Acrosomal Integrity

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