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RESEARCH ARTICLE

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Evaluation of Genetic and Non-genetic Factors Influencing Semen Production Potential in Sahiwal Bulls

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ABSTRACT

Background: Understanding genetic and non-genetic factors that influence the quality and quantity of semen production plays a crucial role in determining the reproductive efficiency and genetic progress within the Sahiwal breed. The objective of this study was to assess the impact of genetic and non-genetic factors on the semen production potential of Sahiwal bulls.

Methods: Semen ejaculate data, including volume (VOL), sperm concentration per milliliter (CONC), total sperm count per ejaculate (TS), initial motility (INIT) and post-thaw motility (PT), as well as semen quality traits such as hypo-osmotic swelling test (HOST) and acrosome integrity of frozen semen (AIFS), were obtained from 52 Sahiwal bulls. The data spanned from January 2011 to December 2018 and were collected at BAIF's frozen semen station in Jind, Haryana, India. To ensure data reliability, observations beyond the mean±4 standard deviations (S.D.) were identified as outliers and subsequently excluded from the study. Two key factors, namely the season of semen collection (winter, summer and monsoon) and the age of bulls at collection (categorized into 12-month intervals ranging from less than 36 months to 156 months), were considered influential factors affecting semen characteristics. To investigate the correlation between bulls (genetic + permanent environmental correlation) and semen parameters, a repeatability animal model was employed, treating the bull as a random effect. The statistical analysis was conducted using WOMBAT software.

Result: The findings revealed that the season of semen collection had a highly significant effect ($P<0.01$) on VOL, CONC, TS and PT. However, INIT, HOST and AIFS were not influenced ($P>0.05$) by the season of collection. Furthermore, age at semen collection significantly affected all semen characteristics. VOL and TS exhibited an increasing trend until 72 to 84 months, followed by a decline until 96 to 108 months.

Key words: Repeatability animal model, Sahiwal, Semen quality.

INTRODUCTION

The breeding and reproductive performance of livestock are of utmost importance in ensuring sustainable agricultural practices and meeting the growing global demand for animal products. In bovine reproduction, the quality and quantity of semen play a crucial role in determining the reproductive efficiency and genetic progress within a breed. As one of the most important indigenous cattle breeds in India, the Sahiwal breed has gained recognition for its adaptability, heat tolerance and high milk production potential (Bhakat *et al*., 2011 and Kumar *et al*., 2018).

The genetic and non-genetic factors that influence semen production potential in Sahiwal bulls are multifaceted and complex. Understanding these factors and their interplay is essential for the effective management of breeding programs and the enhancement of reproductive outcomes. Both genetic factors along with non-genetic factors can significantly impact semen quality and quantity. While genetic factors are undeniably important, non-genetic factors must not be overlooked. Environmental and management factors can exert a significant influence on semen quality and fertility, potentially overshadowing the genetic contributions (Bhakat *et al*., 2011; Bhave *et al*., 2020 and 2021). Furthermore, the effect of various seasonal changes on semen quality and fertility warrants a thorough investigation, considering the Sahiwal breed's origin and adaptation to tropical environments.

In this manuscript, we present a comprehensive evaluation of the genetic and non-genetic factors influencing semen production potential in Sahiwal bulls. By integrating genetic analyses, phenotypic assessments and environmental considerations, we aim to provide a holistic understanding of the factors contributing to the reproductive

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performance of this valuable breed. Our findings will contribute to the scientific knowledge surrounding Sahiwal bull reproduction. Overall, this study highlights the significance of both genetic and non-genetic factors in determining semen production potential in Sahiwal bulls. By unraveling the intricate interplay between genetics and the environment, we can pave the way for sustainable and efficient breeding programs, ensuring the continued success and preservation of the Sahiwal breed in the face of evolving agricultural demands and challenges. With this the study aimed at assess the impact of genetic and non-genetic factors on the semen production potential of Sahiwal bulls.

MATERIALS AND METHODS

Data

Semen ejaculate information on semen production traits *viz*. ejaculate volume (VOL): 13186, sperm concentration per ml (CONC): 13084, total sperm per ejaculate (TS): 13130, initial motility (INIT): 13077 and post-thaw motility (PT): 12157; and semen quality traits *viz*. hypo-osmotic swelling test (HOST): 601 and acrosome integrity of frozen semen (AIFS): 594,belonging to 52 Sahiwal bulls, available from January 2011 to December 2018 at BAIF's frozen semen station, Jind, Haryana, India, were used for the study.

Farm location and climate

The BAIF frozen semen station is situated within the Haryana state, India, specifically positioned on the periphery of Jind city (29.3159°N, 76.4896°E) at an elevation of 227 meters above the mean sea level. This locale experiences three distinct climatic seasons, namely, summer (March-May), monsoon (June-October) and winter (November to February). The average minimum temperature throughout the year ranged from 6.4°C in the month of January to 17.3°C in October. Conversely, the average maximum temperature exhibited fluctuations from 29.1°C in November to 40.9°C in May. With respect to atmospheric moisture, the mean relative humidity demonstrated variability, ranging from 23% in April to 78% in August. As for precipitation, the region receives an average annual rainfall of 536 mm, primarily concentrated during the months of July to September. Monthly precipitation averages fluctuated between 1 mm (April) and 178 mm (August).

Traits studied and influencing factors

The semen traits included in the study were VOL (ml), CONC (10^9 /ml), INIT and PT (% of motile sperms after dilution and before/after thawing), TS (10^9 /ejaculate, as the product of volume and sperm concentration), HOST and AIFS (%). The observations beyond mean±4 standard deviations (S.D.) were considered as outliers and removed from the study. The various factors influencing semen characteristics considered in the study were season (winter, summer and monsoon) and age (classified as 12 months interval class, from less than 36 months to 156 months) at collection.

Semen processing for quality control

In this scientific study, a standardized procedure was followed to collect and evaluate bull semen for artificial insemination purposes. The bulls involved in the study were washed and cleaned early in the morning prior to semen collection. The person responsible for handling and collecting semen from a particular bull also took care of the entire group of bulls. To ensure proper sexual stimulation, dummy bulls were used to simulate mounting behaviour. Each bull was given 2 to 3 false mounts before the actual semen collection took place. The duration between these false mounts varied among the bulls. Semen collection was performed using individual artificial vaginas according to a standard procedure outlined in the Government of India (GoI Report, 2022). Following collection, the ejaculate volume was recorded and the semen tube was kept in a water bath at 37°C. Sperm concentration was estimated using a digital photometer from IMV Technologies. The initial motility, specifically the percentage of progressive motile sperm, was subjectively assessed using diluted semen. Any ejaculates that did not meet the minimum criteria set by standards were excluded from production, although the corresponding data was still considered for analysis purposes. After the initial assessment, 0.25 ml semen doses were prepared, each containing 20 $\times$ 10^6 sperm per dose. These doses were sealed and labelled using the IS4 instrument from IMV Technologies. The semen straws were then cooled gradually to 4°C over a period of 3 hours and subsequently frozen step by step to -140°C for 7 to 8 minutes using a programmable freezer from IMV Technologies. Finally, the semen straws were submerged and stored in liquid nitrogen at -196°C for long-term preservation. Post-thaw motility assessment was conducted after 24 hours of freezing. Different technician carried out initial and post-thaw motility assessment separately. The evaluations of motility were performed using a phase contrast microscope (Nikon ECLIPSE E400, Tokyo, Japan). After cryopreservation, random samples from each bull's batch of semen doses underwent quality control testing. These tests were conducted once every three months, allowing each bull to be tested four times a year. The hypo-osmotic swelling test (HOST) was conducted by mixing 0.1 ml of frozen-thawed semen with one ml of a hypo-osmotic solution, followed by incubation at 37°C for at least 30 minutes (Jeyendran *et al*., 1984). The resulting stained smear was observed under a phase contrast microscope to assess the appearance of "tail curling" in the sperm. The integrity of the acrosome was tested using Giemsa staining on frozen semen. The cut-off values for these tests were set at >40% for the HOST and >65% for the acrosome integrity test.

Statistical analysis

The semen production records were subjected to statistical analysis using mixed model analysis using "*lme4*" function using "*lmerTest*" package in R statistical software (R Core

Team, 2022). The ANOVA and fixed effect were estimated using "*anova*" and "*summary*" functions. The model for the analysis is given below:

$Y_{ijkl} = S_i + A_j + U_k + e_{ijkl}$

Where:

Y_{ijkl} = Semen production and quality traits.

S_i = Fixed effect of the i^{th} season of semen collection (i=1 to 3).

A_j = Fixed effect of the j^{th} age at semen collection (j=1 to 11).

U_k = Random effect of the k^{th} bull (r= 1 to 52) , supposed to follow a normal distribution with mean 0 et and variance σ^2_b.

e_{ijkl} = Random residual associated with each observation, supposed to follow a normal distribution with mean 0 and variance σ^2_e.

To study the bull correlation (Genetic + Permanent environmental correlation) on the semen parameters, a repeatability animal model with the bull as a random effect was used in WOMBAT software (Meyer, 2007).

RESULTS AND DISCUSSION

The overall mean and fixed effect solution for all semen production and quality parameters of the Sahiwal bulls are presented in Table 1.

The season of semen collection had a very high significant (P<0.01) effect on VOL, CONC, TS and PT while INIT, HOST and AIFS didn't influenced (P>0.05) by the season of collection. The winter season was considered as a reference class while the other classes within the season effect were compared against winter. The CONC and TS were higher (by 0.11 and 0.59 billion per ml) in summer than winter season and declined in monsoon, while VOL declined slightly in summer (by 0.05 ml). The differences among the season of collection for the PT were very minute (barely 0.22 per cent). The average period of spermatogenesis and epididymal maturation is 65 days. The influence of seasonal variation during the sensitive stages of spermatogenesis and epididymal maturation may have affected on CONC and subsequently. In simple words, the sperm output in the ejaculate is the reflection of environmental conditions to which the bulls were exposed about 2 months prior to the collection day. A similar seasonal effect was reported by Fuerst-Waltl *et al*. (2006), Murphy *et al*. (2018), Bhave *et al*. (2020) and Bhave *et al*. (2021) in Austrian Simmental, Holstein Friesian, Gir and other Indian cattle breed bulls, respectively. Despite the statistically significant effect of season on VOL, the difference was found to be very low and not affected by spermatogenesis process as seminal fluid which contributes to the VOL produced on the day of collection which is consistent with the findings of Fuerst-Waltl *et al*. (2006). The significant effect of season on PT could be due to large sample size.

All the semen characters were significantly affected by age at semen collection. The VOL and TS showed increasing trend up to 72 to 84 months and declined till 96 to 108 months. Both the traits showed another increment just to decline sharply at the end. Mathevon *et al*. (1998), Fuerst-Waltl *et al*. (2006), Bhave *et al*. (2020) and Bhave *et al*. (2021) found similar findings for volume and total sperm in Holstein, Austrian SimmentalGir and other Indian cattle breeds respectively. It was also noted by Almquist, (1978) that total sperm output increased as a result of an increase in the size of testes until 5 to 6 years after puberty. While

Table 1: Fixed effect of season and age at collection on semen traits in the Sahiwal bulls.

Levels of factors	VOL (ml)	CONC (10^9/ ml)	TS (10^9/ ejaculate)	INIT (%)	PT (%)	HOST (%)	AIFS (%)
Overall mean±S.D.	5.99±1.98	1.46 ±0.65	8.67 ±4.45	76.34 ±9.08	55.94±3.23	58.52 ±10.61	76.17±7.96
Season of collection	**	**	**	NS	**	NS	NS
Winter	0*	0*	0*	0*	0*	0*	0*
Summer	-0.05±0.04	0.11± 0.01	0.59±0.09	0.25±0.20	-0.22±0.08	-0.61±1.04	0.06± 0.61
Monsoon	-0.23±0.03	-0.07±0.01	-0.70±0.08	0.01±0.17	-0.18±0.07	-0.39±0.88	0.12±0.52
Age at collection	**	**	**	**	*	**	**
< 36 months	0*	0*	0*	0*	0*	0*	0*
>36 to 48 months	0.38±0.07	0.15±0.03	1.25±0.18	0.29±0.40	0.36±0.15	3.81±1.86	2.28±1.11
>48 to 60 months	0.32±0.08	0.14±0.03	1.15±0.20	1.35±0.44	0.52±0.17	7.54±1.95	6.28±1.17
> 60 to 72 months	0.77±0.08	0.12±0.03	1.69±0.20	2.33±0.44	0.43±0.17	6.67±2.00	7.34±1.21
> 72 to 84 months	0.60±0.08	0.12±0.03	1.51±0.21	3.07±0.46	0.35±0.17	7.33±2.04	7.22±1.24
> 84 to 96 months	0.45±0.09	0.09±0.03	1.08±0.23	0.64±0.52	0.32±0.20	6.03±2.27	8.17±1.39
> 96 to 108 months	0.28±0.11	0.14±0.04	1.30±0.26	-0.56±0.58	0.07±0.21	8.70±2.42	11.22±1.47
> 108 to 120 months	0.68±0.12	0.08±0.04	1.42±0.29	-3.43±0.63	0.09±0.23	8.39 ±2.63	11.33±1.60
> 120 to 132 months	0.53±0.12	0.09±0.04	1.31±0.31	-3.81±0.68	0.36±0.25	14.92±2.71	14.05±1.65
> 132 to 144 months	0.47±0.13	0.15±0.05	1.50±0.33	0.38±0.72	0.02±0.26	11.85±3.05	12.61±1.85
> 144 to 156 months	-0.02±0.14	0.13±0.05	0.77±0.35	-4.70±0.79	-0.32±0.30	12.90±3.12	11.81±1.89

VOL: Ejaculate volume (ml); CONC: Sperm concentration (10^9/ ml); TS: Total sperm (10^9/ ejaculate); INIT: Initial motility (%); PT: Post thaw motility (%); HOST: Hypo osmotic swelling test (%); AIFS: Acrosome integrity of frozen semen.

Significance level: **p<0.01; *p<0.05; NS: p>0.05.

Table 2: Repeatability and correlation estimates of semen traits in Sahiwal bulls.

Traits	VOL	CONC	TS	INIT	PT	HOST	AIFS
VOL	0.35±0.04	-0.33±0.13	0.29±0.13	0.11± 0.14	0.26±0.14	0.01± 0.16	-0.22±0.15
CONC	-0.11± 0.05	0.35±0.05	0.79±0.06	0.26±0.14	-0.06±0.15	0.11±0.17	0.14±0.15
TS	0.33±0.04	0.58±0.02	0.27±0.04	0.36±0.13	0.13±0.15	0.12±0.16	-0.03±0.16
INIT	0.07±0.03	0.11± 0.04	0.15±0.03	0.17±0.03	0.68±0.08	0.09±0.17	-0.08±0.16
PT	0.05±0.02	-0.01±0.03	0.01±0.02	0.09±0.02	0.07±0.01	0.06±0.18	-0.03±0.17
HOST	0.02±0.06	0.01±0.07	0.02±0.06	0.04±0.05	0.01±0.04	0.39±0.06	0.86±0.05
AIFS	-0.09±0.07	0.06±0.08	-0.001±0.07	-0.04±0.05	-0.03±0.04	0.49±0.06	0.66±0.05

VOL: Ejaculate volume; CONC: Sperm concentration per ml (Billion); TS: Total sperm per ejaculate (Billion); INIT: Initial motility (%); PT: Post thaw motility (%); HOST: Hypo osmotic swelling test (%); AIFS: Acrosome integrity of frozen semen.

Diagonal values: Repeatability estimates; Upper diagonal values: Bull correlation; Lower diagonal values: Phenotypic correlations.

increase in total sperm was due to subsequent rise in VOL and not due to CONC from the present study is consistent with the findings of Foote *et al*. (1977). Another increment observed in total sperm could have arisen due culling of 45 bulls out of 52 which is approximately 86 per cent while keeping only 7 bulls. The bulls with improved performance were retained where the bull with high demand in the field (possibly due to better fertility) was kept for an extended period. While the CONC declined slowly as the age progressed. INIT and PT followed trend of the CONC which showed progressively declined with the age except for the fact that the differences among the age classes were very small ranging between 0.29 to 4.70 per cent for the INIT and 0.02 to 0.52 per cent for PT. On the contrary, it was observed that HOST and AIFS increased gradually with the age.

The genetic parameters for all semen attributes are presented in Table 2. The repeatability of semen traits ranged from 0.07 for PT to 0.66 for AIFS. The moderate repeatability estimates were observed in VOL, CONC and HOST while low estimates were observed especially in INIT and PT while the high estimates were found in AIFS. The bull correlation ranged from -33 to 0.86. VOL and CONC had negative bull correlation while CONC shared a high bull correlation with TS. Motility traits (INIT and PT) were moderately correlated with VOL, CONC and TS, while both the motility traits shared high correlation among each other. The bull correlations between the production traits (VOL, CONC, TS, INIT and PT) and quality traits are very low irrespective of their direction except for the CONC-TS which was very high. HOST and AIFS showed very high bull correlation. All the phenotypic correlations are lower and in similar direction with their respective bull correlations.

The repeatability and bull correlation estimates are regarded as the higher limit for the heritability and genetic correlations, respectively. The heritability and genetic correlations could not be estimated due to limited availability of genetic relationship or pedigree of the bulls. There is death of availability of literatures or studies on genetic parameters of semen traits in Sahiwal bulls. The repeatability estimates of PT and AIFS are in close accordance with report of Bhave *et al*. (2020) in Gir bulls which were maintained under same management conditions. The semen traits with moderate to high repeatability could explain the future performance of the bulls with certain accuracy.

CONCLUSION

The present study leads to the conclusion that the observed seasonal fluctuations in semen parameters can be attributed to the impact of climatic conditions during the vulnerable stages of spermatogenesis. Furthermore, the investigation revealed an age-related pattern in semen characteristics, indicating that individual bulls exhibited variations in measured traits as they advanced in age at the time of collection. The assessment of repeatability and bull effects offers valuable insights into explaining the future performances of bulls with a reasonable level of accuracy. Ultimately, understanding the genetic and environmental factors that influence semen production can assist an artificial insemination (AI) facility in achieving a high output of semen with favorable quality, thereby optimizing profitability.

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Conflict of interest

The authors declare that they have no competing interests.

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