



International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

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www.agronomyjournals.com

2024; SP-7(2): 40-43

Received: 18-11-2023

Accepted: 24-12-2023

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Effect of seasons on crossbred bull on semen characteristics and seminal plasma enzymes of fresh semen

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DOI: <https://doi.org/10.33545/2618060X.2024.v7.i2Sa.333>

Abstract

The objective of the current research was to investigate the impact of various seasons (Winter, Summer, and Monsoon) on seminal characteristics and seminal plasma enzymes in fresh semen from crossbred breeding bulls. Ninety-six ejaculates were collected through the artificial vaginal method from ten Holstein Friesian × Gir breeding bulls with 50% and 75% exotic inheritance over a twelve-month period, segmented into three different seasons. Semen analysis was conducted on fresh semen, revealing significant variations in semen ejaculate volume and mass activity across different seasons. Notably, the semen ejaculate volume was significantly higher during the summer compared to the winter and monsoon seasons, although the difference between winter and monsoon was not statistically significant. Parameters such as density, sperm concentration, and pH remained comparable throughout different seasons. Furthermore, the values of seminal plasma enzymes, including ALP, ACP, and CPK activity in fresh semen samples during different seasons, exhibited statistical significance ($p < 0.05$), while other parameters showed no significant differences. In conclusion, the study underscores substantial differences in seminal characteristics and seminal enzyme activities among seasons in Holstein Friesian crossbred breeding bulls in fresh semen samples. These findings suggest that bulls with exotic inheritance may be more susceptible to changes in environmental temperature. Thus, it is crucial to implement proper management practices to mitigate the impact of heat stress, aiming to enhance semen quality and improve fertility in crossbred breeding bulls.

Keywords: Crossbred bulls, seasons, seminal characteristics, seminal enzymes

Introduction

Seminal plasma, while physiologically significant, comprises a complex molecular composition primarily constituted by various proteins that are not yet fully understood. In recent years, there has been notable progress in the identification, isolation, and characterization of several seminal plasma proteins. Research indicates that the protein composition of seminal plasma varies among species, and there is growing evidence that certain seminal plasma proteins are linked to fertility in diverse species (Asadpour, 2012) [2].

Earlier studies conducted on seminal characteristics and seminal plasma have been conducted individually (Dalton *et al.*, 2012) [6]. However, in the available literature no report has been found where all these variables or parameters were studied together in the animals. This would help to judge/ assess male fertility accurately. The information on seminal enzymes is sparse and limited in fresh semen in the crossbred bulls with respect to seasonal variation. The current study has been initiated with specific objectives aimed at determining the concentration of seminal attributes, encompassing both macroscopic and microscopic evaluations, as well as seminal plasma enzymes in crossbred breeding bulls. The focus is on exploring potential variations in these parameters across different seasons within the Konkan region.

Materials and Method

The study was conducted at the Department of Veterinary Biochemistry, Bombay Veterinary College, Parel, Mumbai – 400 012.

The macro and microscopic evaluation of semen samples took place at the Frozen Semen Laboratory, Development Corporation Konkan Limited, Unit No. 16, Aarey Milk Colony, Goregaon (E), Mumbai – 400 065. Seminal plasma enzyme estimations were performed using enzyme kits, and the facilities for this analysis were provided by Unique Biodiagnostics Laboratory, Parel, Mumbai - 400 012.

Eight apparently healthy Holstein Friesian crossbred bulls, with 50% to 75% exotic inheritance (Holstein Friesian X Gir), were selected for the study. These bulls were housed at Unit No. 16, Development Corporation of Konkan Limited, Aarey Milk Colony, Goregaon (E), Mumbai – 400 065, with an age range of 4 to 8 years. Semen samples were obtained twice a week through artificial vagina over a period of about one year, collecting samples once a month. A total of 96 samples were collected during the experimental period.

Immediately after collection, each semen sample was placed in a water bath at 37 °C and evaluated for various physical characteristics. Semen quality measures were conducted following the methods outlined by Borsekar (1980). Both macroscopic and microscopic evaluations of semen samples were performed using traditional methods.

Seminal plasma was separated by centrifuging semen from all bulls at 5000 rpm for 10 minutes and stored at –20 °C until use. The analysis of seminal plasma enzymes was carried out on the auto analyzer Prietest Touch (Robonik India). Statistical analysis of the data followed the methodology by Snedecor and Cochran (1994) [16] using a completely randomized design, and differences in means were assessed using the critical difference (CD) test.

Results and Discussions

The mean values of macroscopic (volume, colour, density, consistency) and microscopic (sperm concentration, mass activity and pH), characteristics of fresh semen samples during varying seasons is presented in Table 1

Semen Evaluation

Macroscopic evaluation

The average volume of fresh semen ejaculate was highest in summer, lowest in winter and intermediate in monsoon. The difference in the ejaculate volume between summer and winter was statistically significant. The volume of semen was higher in monsoon than winter but the difference was not statistically significant. Seasonal effects on semen production are influenced by a myriad of factors, including ambient temperature, humidity, day length, and food quality. Consequently, the complexities surrounding seasonal variation in semen production remain not

fully comprehended.

The colour of the fresh ejaculated semen samples was on an average white in colour during winter and creamy white in colour during summer and monsoon season, respectively. There was no significant effect of season on colour of semen (Bhakat *et al.*, 2014 and Alragubi *et al.*, 2015) [4, 1]. Sarder (2007) [13] reported significant effect on colour of semen due to different seasons. The consistency of the fresh ejaculated semen was on an average thick during winter, summer and monsoon seasons, respectively. Consistency of fresh semen sample was not affected by the season in the present study.

The average density of fresh semen was highest during monsoon season and lowest during summer. The differences in the values of density did not differ statistically. Sarder *et al.* (2007) [13] in a study on 71 breeding bulls (Holstein Friesian, Sahiwal and Local crossbred bulls) reported that the density of semen collected from these bulls was 3.57±0.97 in spring, 3.59±0.82 in summer, 3.59±0.78 in autumn and 3.49±0.77 in winter. The difference in the values between winter and other seasons were statistically significant.

The average semen pH of fresh semen was on higher side (6.78±0.03) in winter and on lower side (6.66±0.37) during monsoon season. The small differences in the values of semen pH did not differ significantly. Bhakat *et al.* (2014) [4] reported pH of semen as 6.93±0.04, 6.90±0.03 and 6.88±0.03 during summer, rainy and winter season in Karan Fries crossbred bulls. The differences in values of pH however were statistically non-significant. This finding is in agreement with the results of present study.

Microscopic evaluation

The average mass activity of fresh semen ejaculate was found to be highest during the monsoon season and lowest in the summer season. However, the slight differences in mass activity values between seasons were not deemed statistically significant. This aligns with the observations of Mathur *et al.* (2002) [12] in Frieswal bulls and Helbig *et al.* (2007) [9] in American bison, both of whom reported no significant seasonal variations in mass activity. Their findings resonate with the results obtained in the present study. In the case of sperm concentration (measured in millions/ml) in fresh semen ejaculate from Holstein Friesian X Gir crossbred bulls, the average was highest during the winter season, lowest in the summer season, and intermediate in the monsoon season. Notably, the differences in sperm concentration values between the winter and summer, as well as the summer and winter seasons, were statistically significant ($p < 0.05$).

Table 1: Seminal attributes of fresh semen during varying seasons

Seminal Characteristics	Seasons		
	Winter	Summer	Monsoon
Volume of ejaculate	8.57±0.44 ^b	10.20±0.22 ^a	9.10±0.43 ^b
Colour	Creamy	Milky white	Creamy
Density	3.38±0.06	3.24±0.12	3.34±0.09
Consistency	Thick	Thick	Thick
Sperm concentration	1225.35 ±24.42	1110.72±30.02	1140.08±19.63
Mass activity	3.17±0.14 ^a	3.10±0.12 ^b	3.28±0.24 ^b
pH	6.88±0.04	6.62±0.03	6.68±0.38

The average concentration of various enzymes in fresh semen samples of Holstein Friesian X Gir crossbred bulls during winter, summer and monsoon season is presented in Table 2.

Seminal plasma aspartate transaminase: The average

concentration of aspartate transaminase (AST) in fresh semen samples exhibited an ascending order during the winter, monsoon, and summer seasons, respectively. The highest value of the aspartate transaminase enzyme was reported during the

summer, while the lowest was observed during the winter season. The differences in AST values between winter and summer, as well as winter and monsoon, were statistically significant ($p < 0.05$), while the differences between summer and monsoon in fresh semen samples were not statistically significant.

Transaminases present in semen are involved in oxidative metabolic pathways, providing energy substrates to sperm cells. Khawaskar *et al.* (2012) [10] investigated seasonal variations in

AST concentration in the seminal plasma of Surti buffalo bulls and reported that AST activity was lowest during the monsoon season. In the present study, the concentration of AST in seminal plasma was highest during the summer season (223.83 ± 12.92 U/L). These findings are consistent with those of Rajoriya *et al.* (2013) [15], who reported a significant difference in AST activity in seminal plasma in fresh semen between the winter and summer seasons in Tharparkar bulls.

Table 2: Seminal plasma aspartate transaminase enzyme concentration (U/L) during different seasons of the year; in fresh, diluted and frozen semen sample.

Seminal plasma enzymes (IU/L)	Seasons			Level of sign.
	Winter	Summer	Monsoon	
Aspartate transaminase	149.67 \pm 18.82 ^a	222.88 \pm 13.82 ^{ab}	198.65 \pm 18.08 ^{ab}	*
Alkaline phosphatase	1435.22 \pm 218.39 ^a	2685 \pm 228.96 ^{ab}	1698 \pm 146.52 ^{ab}	*
Acid phosphatase	123.56 \pm 2.65 ^a	312.65 \pm 23. ^b	130.54 \pm 3.812 ^a	*
Lactate Dehydrogenase	634.22 \pm 30.23 ^a	1031.71 \pm 250.60 ^b	688.44 \pm 38.82 ^c	*
Creatine phosphokinase	238.63 \pm 223.65 ^a	365 \pm 194.88 ^{ab}	305.69 \pm 48.72 ^{ab}	*
Glutamyl transferase	1255.65 \pm 116.88 ^a	1488 \pm 98.15 ^a	1385.12 \pm 107.80 ^a	NS

Mean with common superscript do not differ significantly

Seminal plasma alkaline phosphatase

The average concentration of alkaline phosphatase (ALP) in fresh semen was observed to be highest during the summer season and lowest during the winter season. The differences in ALP values between the winter and summer, as well as the summer and monsoon, in fresh semen samples were statistically significant ($p < 0.05$). Notably, no significant variation in the content of alkaline phosphatase enzyme was reported in Surti buffalo bull semen during summer, monsoon, and winter seasons (Reddy and Raja, 1980) [14]. Additionally, Khawaskar *et al.* (2012) [10] reported a low concentration of alkaline phosphatase in the seminal plasma of Surti buffalo bulls during the summer. The higher value of ALP in the seminal plasma during the summer season in the present study is consistent with the findings of Dhama and Kodagali (1990) [8] in buffalo.

Seminal plasma acid phosphatase

The average concentration of acid phosphatase (ACP) in fresh semen samples showed a descending order during the summer, monsoon, and winter seasons. The highest value of the acid phosphatase enzyme was reported during the summer, while the lowest was observed during the winter season. The differences in ACP values between winter and summer, as well as summer and monsoon seasons, were statistically significant ($p < 0.05$). These findings align with the results of the study by Khawaskar *et al.* (2012) [10], which demonstrated seasonal variation in the seminal plasma enzyme profile of Surti buffalo bulls, with a higher concentration of ACP in the summer season and the lowest in the monsoon season. Notably, no significant variation in acid phosphatase in Surti buffalo bull semen during different seasons was reported by Reddy and Raja in 1980 [14].

Seminal plasma lactate dehydrogenase

The average concentration of lactate dehydrogenase (LDH) in fresh semen was found to be highest during the summer season and lowest during the winter season. However, the differences in LDH values between winter and summer, winter and monsoon, and summer and monsoon in fresh semen samples were comparable, and the differences were non-significant. A study by Dhama *et al.* (1986) [7] on Surti buffaloes during three different seasons reported LDH values of 395.94 ± 10.15 , 400.51 ± 10.04 , and 64.69 ± 16.85 IU/L during winter, summer,

and monsoon seasons, respectively. In the present study, a higher concentration of LDH was reported in the summer season, and the lowest was observed in the winter season in fresh semen samples.

Seminal plasma creatine phosphokinase

The average concentration of seminal plasma creatine phosphokinase (CPK) enzyme in fresh semen exhibited the highest value during the summer season and the lowest during the winter season. Notably, the differences in CPK values between winter and summer, as well as summer and monsoon seasons in fresh semen samples, were statistically significant ($p < 0.05$). It is worth mentioning that creatine phosphokinase levels in human sperm are considered an objective biomarker of sperm maturity and fertilizing potential.

Seminal plasma γ -glutamyltransferase

The average concentration of seminal plasma γ -glutamyltransferase (GGT) enzyme was observed to be highest during the summer season and lowest during the winter season. However, the differences in the values of the GGT enzyme between winter and summer, winter and monsoon, and summer and monsoon in fresh semen samples were not statistically significant. Notably, GGT plays a major role in the glutathione system, which is involved in protecting spermatozoa against oxygen radicals (Awadallah *et al.*, 2003) [3].

Conclusion

The present study draws the conclusion that there are significant differences in seminal characteristics and seminal enzyme activities among seasons in Holstein Friesian crossbred breeding bulls, particularly in fresh semen samples. These findings underscore the vulnerability of breeding bulls with exotic inheritance to changes in environmental temperature. Consequently, it is recommended that proper management practices be implemented to mitigate the consequences of heat stress, with the aim of enhancing semen quality and ultimately improving fertility in these animals.

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