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Influence of seasons on cow bull seminal plasma testosterone and proteins

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Abstract
The current research aimed to evaluate the impact of seasons (winter, summer, and monsoon) on seminal proteins and testosterone levels in Holstein Friesian × Gir crossbred breeding bulls. A total of 96 ejaculates were collected using the artificial vaginal method from eight Holstein Friesian × Gir breeding bulls with 50% and 75% exotic inheritance over a one-year period, categorized into three seasons. Semen analysis was conducted on both fresh semen and semen diluted with the standard TEYC extender. The seminal plasma testosterone concentration in fresh semen samples exhibited statistically significant differences across different seasons ($p<0.05$). The highest concentration of seminal plasma testosterone was recorded during the summer season, while the lowest concentration was noted during winter. Seminal plasma proteins, ranging from 10 to 205 kDa, were observed in fresh, diluted, and frozen semen samples. Although all bulls displayed the same pattern of protein bands, variations in protein concentrations were attributed to seasonal differences. In conclusion, the study revealed significant variations in some seminal plasma testosterone levels among seasons in Holstein Friesian crossbred breeding bulls in both fresh and cryopreserved semen samples. These findings highlight the vulnerability of these animals to changes in environmental temperature, emphasizing the need for effective management practices to mitigate the impact of heat stress and enhance fertility in breeding bulls with exotic inheritance. A better cry opreservative should be used to preserve the semen of the crossbred animals.

Keywords: Cow bull, proteins, seasons, seminal plasma, testosterone

Introduction
The organic constituents present in seminal plasma play a crucial role in sustaining sperm metabolism, maintaining pH, osmolarity, and contributing significantly to sperm function in mammals, as highlighted by Maxwell *et al.* in 2007. When seminal plasma concentration is reduced through processes like dilution or washing, a phenomenon known as the 'dilution effect,' it can lead to a decline in ejaculated spermatozoa's motility, metabolic activity, Fertilizing capacity, and even result in cell death, as described by Mann in 1954. This dilution effect has a substantial impact on various sperm structures, including membrane destabilization, which may ultimately lead to the demise of sperm cells.

Earlier studies conducted on seminal attributes like sperm motility, sperm morphology, seminal plasma proteins, testosterone concentration in serum and seminal plasma have been conducted individually (Dalton *et al.*, 2012) [3]. However, in the available literature no report has been found where seminal plasma protein and testosterone were studied together in the animals. This would help to predict/assess male fertility accurately. The information on seminal plasma proteins and testosterone is sparse and limited in fresh and frozen-thawed semen in the crossbred bulls in the Konkan region with respect to seasonal variation. The present study was, therefore, undertaken with the objectives to determine the concentration of seminal plasma proteins and seminal plasma testosterone in crossbred breeding bulls during different seasons in the Konkan region.

Materials and Methods

The research was conducted at the Department of Veterinary Biochemistry, Bombay Veterinary College, Parel, Mumbai – 400 012. Semen samples were sourced from the Frozen Semen Laboratory, comprising eight apparently healthy Holstein Friesian crossbred bulls (Holstein Friesian X Gir with 50% to 75% exotic inheritance). These bulls were housed at Unit No. 16, Development Corporation of Konkan Limited, Aarey Milk Colony, Goregaon (E), Mumbai – 400 065, and were between 4 to 8 years old.

Throughout the experimental period, the animals were maintained under a consistent plane of nutrition and management. The bulls were tethered and granted freedom only during semen collection using an artificial vagina. Each bull received a measured quantity of concentrate mixture daily in accordance with the established schedule. Additionally, paddy straw was provided ad-libitum to meet the animals' bulk requirements. Para grass, ranging from 15 to 20 kg per animal, served as a source of green roughage based on availability. Clean drinking water was made available three times a day at 5.00 am, 2.00 pm, and 6.00 pm. Regular deworming was performed, and the bulls were vaccinated against Foot and Mouth disease, HaemorrhagicSepticaemia, and Brucellosis. The experimental period spanned from October 2016 to September 2017.

Collection of semen sample

Ejaculates were procured in an artificial vagina during the morning hours, specifically from 7:30 am to 9:00 am, twice a week for the current study. Eight crossbred bulls were the subjects of semen collection, spanning from October 2016 to September 2017, with collections occurring once a month, preferably between the 25th and 30th of each month. Over the experimental period, a total of ninety-six samples were obtained from these eight Holstein Friesian crossbred bulls. Upon collection, each semen sample was promptly placed in a water bath at 37 °C and assessed for various physical characteristics. Statistical analysis of the data followed the methodology outlined by Snedecor and Cochran (1994) [12], utilizing a completely randomized design. Differences in means were subsequently evaluated using the critical difference (CD) test.

The seminal plasma testosterone concentration was determined using Beckman Coulter kits. The facility required for conducting the Radioimmunoassay was availed from the Radio Isotope Laboratory, Bombay Veterinary College, Parel, Mumbai – 400 012. The seminal plasma proteins SDS page was conceded out at National facility for Biopharmaceuticals, G. N. Khalsa College Laboratory, Matunga, Mumbai-400 019.

Results and Discussions

Seminal plasma testosterone concentration

The average concentration of seminal plasma testosterone (ng/ml) in fresh semen samples from Holstein Friesian X Gir crossbred bulls during winter, summer, and monsoon seasons is detailed in Table 1 and illustrated in Figure 1.

The ascending order of average seminal plasma testosterone concentrations in fresh semen samples was 0.12 ± 0.02, 0.73 ± 0.06, and 0.92 ± 0.07 ng/ml during winter, monsoon, and summer seasons, respectively. The highest concentration of seminal plasma testosterone (ng/ml) was observed during the summer season, while the lowest concentration was recorded during winter. Significantly, the differences in seminal plasma testosterone values between winter and summer, winter and monsoon, and monsoon and summer were highly significant (p<0.01).

In this study, it was noted that the concentration of seminal plasma testosterone was low during the winter season, significantly increased during summer, and showed an intermediate level during the monsoon. However, these observations lack direct comparison due to the absence of previous reports on Holstein Friesian x Gir crossbred bulls. This aligns with Javed *et al*. (2000) [6], who reported no seasonal influence on seminal plasma testosterone in buffalo bulls. They further noted higher testosterone concentrations in the summer season (1.71 ± 0.86 ng/ml) compared to the winter season (1.06 ± 0.68 ng/ml) in buffalo bulls aged 6 to 10 years. The study also concluded that seminal testosterone is higher in adult bulls and is associated with semen quality.

Table 1: Seminal plasma testosterone concentration (ng/ml) during various seasons of the year in fresh semen sample

Season	Testosterone concentration(ng/ml)
Winter	0.12 ± 0.03^{c}
Summer	0.93 ± 0.07^{a}
monsoon	0.73 ± 0.07^{b}

Seasonal effects on testicular steroids are a common phenomenon observed in wild animals. While the ram has been extensively studied for seasonality in domesticated farm animals (Schanbacher and Ford, 1979; Pelletier *et al*., 1981) [11, 10], the domesticated boar, in contrast to its wild counterpart, is generally believed to be unaffected by seasonal influences on steroid production. A study conducted by Claus (1979) [2] involved the measurement of the steroid 5a-androst-16-en-3-one (commonly known as the "boar-taint steroid") in adipose tissue of four sexually inactive boars over more than two years. The study revealed a significant increase in steroid levels during late autumn and early winter, aligning with the breeding season of the wild boar.

Godfrey *et al*. (1990) [4] investigated the impact of season on serum testosterone concentration in Brahman and Hereford bulls. Their observations indicated no significant influence of season on testosterone levels in the serum of these bulls in Nebraska and Texas, USA. In another study, Berndtson *et al*. (1974) [1] noted that testosterone secretion in stallions is evidently influenced by season, alongside behavioral and seminal changes. The seasonal variation in libido and the activities of the stallion's reproductive tract, both secretory and gametogenic, are partly mediated by the pattern of testosterone secretion. Harris *et al*. (1983) [5] reported in their research that stallions in temperate climates exhibited seasonal variations in reproductive parameters.

It appears that the climatic conditions of a place, season, breed, age, health status, nutrition and management of breeding bulls may influence the concentration of seminal plasma testosterone and quality of semen.

Seminal plasma proteins

The protein profile observed in both fresh and frozen samples of all the experimental bulls were same, there are proteins seen ranging from 10 - 205 kDa in the samples which were well determined by the SDS PAGE. Only the difference in the fresh and frozen samples of various bulls was the concentration of protein. The diluted samples also had proteins from the same range but due to dilution the concentration of proteins had become low and some proteins that had low molecular weight disappeared in SDS PAGE. The low, medium and high molecular weight proteins for

distinct protein bands recognized by SDS PAGE gel which are shown in Fig. A, B and C.

The protein band ranging from 25-37 kDawas seen importantly in all the bull samples but this band was only observed with elevated concentration in months of December to February and also from July to September. These may be proteins, which are accountable for the breeding or may be these are the proteins, which are responsible for the better action of the sperm hence they were expressed more during these months.

In a study conducted by Yue *et al*. (2009) [13], it was found that protein spots within the molecular weight range of 15-20 kDa constituted 41% of the relative intensity on the gel. Other researchers have noted that, within the molecular weight range of 12.5 to 83.9 kDa, protein spots with a molecular weight of <21 kDa exhibited the highest relative intensity when using gradient gels. SDS-PAGE analysis of alcohol-precipitated ram seminal proteins by Bergeron *et al*. (2005) [14] revealed the presence of approximately 25 proteins with molecular masses ranging from 14-120 kDa. Among them, a group of proteins with molecular masses of 15-16 kDa and 22-24 kDa was found to be more predominant.

Additional research by Jobim *et al*. (2005) [15] reported molecular weights ranging from 15-115 kDa, with the most prominent spots being those <30 kDa. Interestingly, the presence of a 205 kDa protein band, not detected in the low fertile group of Jersey bulls and in some of the medium fertile group of Jersey bulls, might be associated with fertility. Yue *et al*. (2009) [13] suggested that the relative content of seminal plasma protein could serve as a crucial index for evaluating ram fertility and semen quality.

In the present study, it was observed that all the bulls exhibited the same pattern of protein bands, with differences noted primarily in the intensity of these bands. Moreover, there was a discernible variation in the concentration of proteins, attributable to seasonal changes. This aligns with the findings of a study by Nandre *et al*. (2013) [9], which investigated the seminal plasma protein profile of Indian Bubalusbubalis using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) during both winter and summer seasons. The 2D-PAGE pattern revealed 42, 29, and 28 protein spots during the winter season and 44, 29, and 29 spots during the summer season for the first, second, and third bulls studied, respectively. To identify expressed protein spots in both seasons, ultra high-performance liquid chromatography-mass spectrometry (UPLC-MS) was employed.

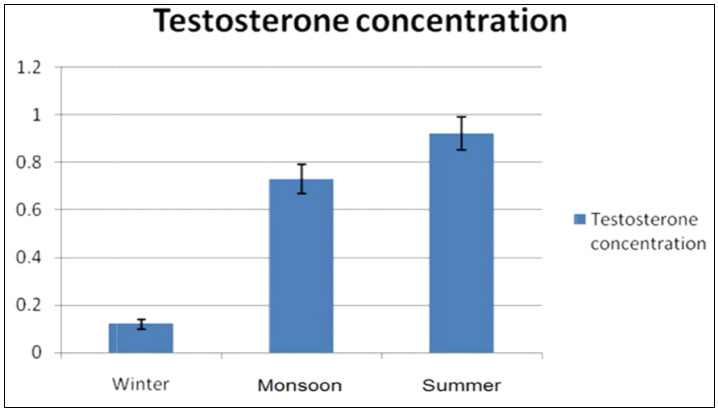
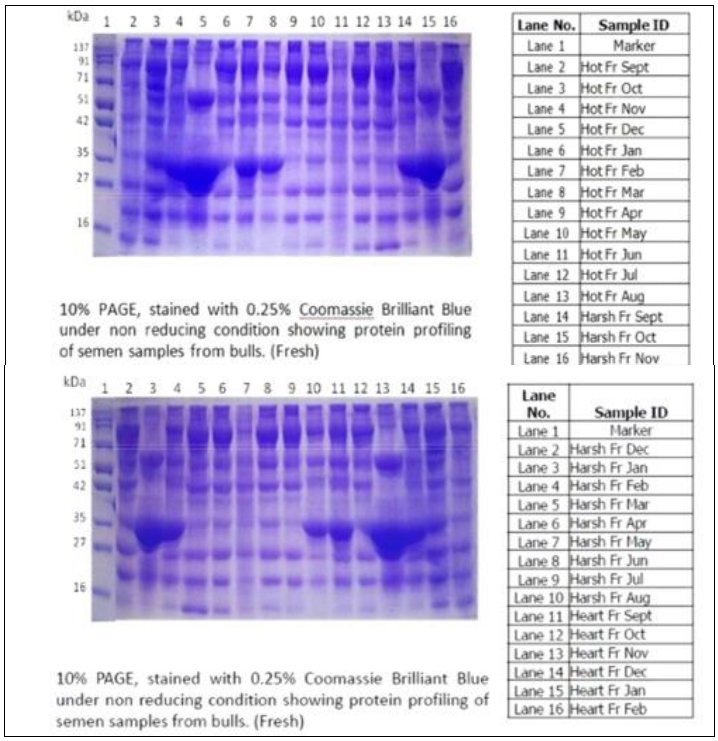
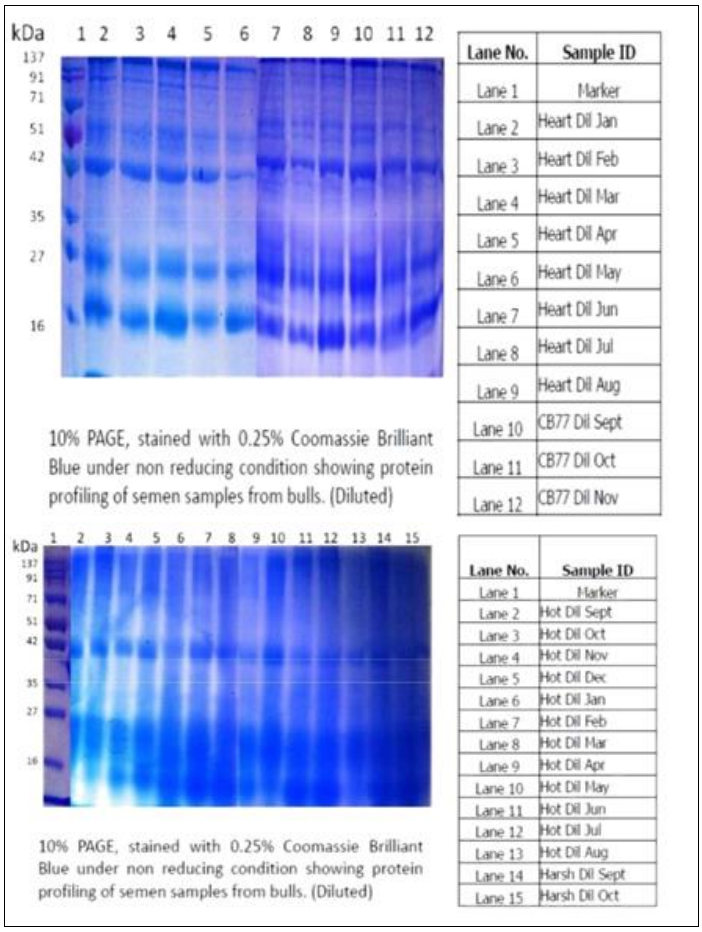


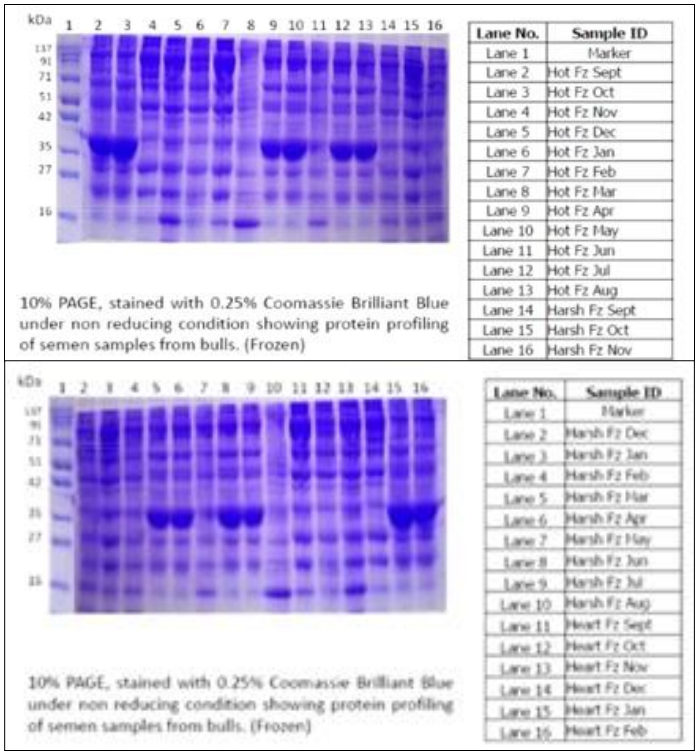
Fig 1: The average seminal plasma testosterone concentration (ng/ml) during various seasons



A



B



C

Fig 2: The low, medium and high molecular weight proteins for distinct protein bands recognized by SDS PAGE gel which are shown in Fig. a, b and c.

Conclusion

The findings of the current study lead to the conclusion that certain seminal plasma testosterone levels exhibited significant variations among seasons in Holstein Friesian crossbred breeding bulls, not only in fresh semen but also in diluted and frozen semen samples. While all bulls displayed a consistent pattern of protein bands, the notable differences lay in the concentration of proteins, primarily attributed to seasonal variations. These observations suggest that animals, particularly those with exotic inheritance, struggle to cope with changes in environmental temperature. Therefore, effective management practices for breeding bulls with exotic

inheritance should be implemented to alleviate the impact of heat stress and enhance fertility. Additionally, the study indicates the need for utilizing improved cryopreservatives for semen preservation in crossbred animals, emphasizing the importance of employing advanced techniques to maintain and enhance semen quality under varying environmental conditions.

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