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Kapale PM
Department of Veterinary Biochemistry, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

AS Nagvekar
Department of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

PM Kekan
Department of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

BN Ramteke
Department of Animal Nutrition, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

NR Dagali
Department of Gynaecology & Obstetrics, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

SD Ingole
Department of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

GM Gadegaonkar
Department of Animal Nutrition, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

Corresponding Author
PM Kekan
Department of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai, Maharashtra, India

Effect of seasons on seminal plasma enzymes and testosterone concentration in Holstein Friesian X Gir crossbred bulls

Kapale PM, AS Nagvekar, PM Kekan, BN Ramteke, NR Dagali, SD Ingole and GM Gadegaonkar

Abstract
The present study was conducted on Holstein Friesian x Gir crossbred (n=8) bulls. The semen samples were collected throughout the year using an artificial vagina at one-month intervals in three seasons (winter, summer, and monsoon). The semen samples collected from the bulls were divided into two parts: one was used for analysis as fresh semen sample, and the second was diluted using an egg yolk extender and cryopreserved for future use. Seminal plasma enzymes like ALP, ACP, CPK activity, and seminal plasma testosterone in fresh semen samples during different seasons were statistically significant (P<0.05). In diluted (pre-freeze) seminal plasma, ALP and CPK activity showed statistically significant differences between seasons. Whereas, GGT in fresh and diluted semen showed a non-significant difference. In frozen (post-thaw) semen samples, the differences in the values of ALP, LDH, CPK and GGT enzyme activities showed significant differences (P<0.05).

Keywords: Holstein Friesian bull, season, seminal attributes, cryopreservation, seminal plasma, enzymes

1. Introduction
The seminal plasma of animals acts as a carrier for spermatozoa from the male testes to their target oocyte. Semen consists of a fluid portion and mediates the chemical function of the ejaculate. Seminal plasma is a complex fluid secreted from the male reproductive tract's testis, epididymis, and accessory sex glands [1]. Large varieties of enzymes are present in the seminal plasma. They include glutamic oxaloacetate transaminase (GOT) or aspartate aminotransferase (AST), glutamic pyruvate transaminase (GPT) or alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and these enzymes are used as good indicators of semen quality because they measure plasma membrane stability of spermatozoa [2]. The enzyme levels in seminal plasma are very important for sperm metabolism as well as sperm function. The possible source of these enzymes is thought to be the testis or epididymis because they show a positive correlation with sperm concentration and a negative correlation with semen volume [3], and the presence of enzyme activities follows reproductive seasonality [4]. Transaminases are located primarily in the mid-piece of spermatozoa [1], whereas LDH is localized in the cytosol and mitochondria of spermatozoa [5]. An adequate evaluation of semen for breeding purposes has tremendous significance, and semen analysis is a valuable diagnostic tool to assess the male's fertility status [6].
AST is an intracellular enzyme of spermatozoa, and an increase in the level of this enzyme in seminal plasma is considered as a determinant of cellular damage [7]. This enzyme is vital for the metabolism and function of spermatozoa and essential for metabolic processes, which provide energy for survival, motility and fertility of spermatozoa [8]. LDH plays a vital role in sperm metabolism, sperm capacitation and fertilization. ALP is primarily of testicular and epididymal origin and, therefore, suitable for differentiation of oligo and azoospermia. Gamma-glutamyl transferase (GGT) plays a major role in the glutathione system that is involved in the protection of spermatozoa against oxygen free radicals [9]. ALP plays a role in keeping spermatozoa quiescent until they are ejaculated and in modulating the acquisition of the fertilizing ability [10]. Creatine phosphokinase (CPK) levels in human sperm are an objective biochemical marker of sperm maturity and fertilizing potential. It has been observed that increased CPK concentrations reflect residual cytoplasm in sperm that was not extruded during late spermiogenesis. Therefore, CPK activities and similar objective biochemical parameters are essential in predicting sperm quality and the fertilizing potential of

oligospermia men [11]. Seminal plasma is one of the richest sources of acid phosphatase (ACP). This enzymatic marker is primarily related to the metabolic function of spermatozoa in ruminants and odd-toed ungulates and prostate diseases in dogs [12].

2. Materials and Methods

The study was conducted on Holstein Friesian x Gir crossbred (n=8) bulls (Holstein Friesian X Gir with 50% to 75% exotic inheritance) aged between 4-8 years maintained at Government Unit No. 16, Konkan Development Corporation (KDCL), Goregaon (East), Mumbai. The semen samples were collected throughout the year using an artificial vagina at one month intervals. According to the region's agro-climatic conditions, the study was undertaken in three seasons, viz., winter, summer and monsoon. The semen samples collected from the bulls were divided into two parts: one was used for analysis as fresh semen sample, and the second was diluted using an egg yolk extender and cryopreserved for future use. Seminal plasma was separated by centrifuging semen of all bulls at 5000 rpm for 10 minutes and was stored at −20°C till use. Using commercially available kits, the seminal plasma enzymes were estimated from all the 96 samples (fresh, diluted and frozen–thawed). Testosterone hormone was estimated by the RIA method. Analysis of data variance was done according to Snedecor and Cochran [13] by using a completely randomized design.

3. Results and Discussion

The mean values of seminal plasma enzymes and testosterone concentration of fresh, diluted and post-thaw semen samples of Holstein Friesian X Gir crossbred bulls during winter, summer and monsoon season is presented in Table 1.

Table 1: The mean ± SE values of seminal plasma enzymes and testosterone concentration of fresh, diluted and post thaw semen samples of Holstein Friesian X Gir crossbred bulls during winter, summer and monsoon season

Seminal plasma parameters	Seasons			
	Winter	Summer	Monsoon	Level of significance
Aspartate transaminase (IU/L)				
Fresh semen	150.571 ± 17.92^{d}	223.83 ± 12.92^{cd}	200.55 ± 11.06^{cd}	*
Diluted semen	224.405 ± 19.65^{cd}	318.315 ± 47.98^{c}	248.125 ± 16.70^{cd}	*
Post thaw semen	642.698 ± 5.65^{b}	915.75 ± 79.67^{a}	716.703 ± 81.63^{b}	*
Alkaline phosphatase (IU/L)				
Fresh semen	1465.18 ± 176.38^{f}	2696.81 ± 275.70^{def}	1686.65 ± 194.98^{ef}	*
Diluted semen	3075.62 ± 372.43^{de}	3553.78 ± 361.55^{de}	3252.00 ± 247.50^{d}	*
Post thaw semen	4866.62 ± 414.34^{c}	4866.62 ± 414.34^{c}	10807.93 ± 1387.51^{a}	*
Acid Phosphatase (IU/L)				
Fresh semen	125.15 ± 2.55^{d}	327.59 ± 23.52^{c}	128.18 ± 1.95^{d}	*
Diluted semen	373.81 ± 19.20^{bc}	423.00 ± 25.50^{b}	369.96 ± 27.96^{bc}	*
Post thaw semen	408.21 ± 19.83^{bc}	602.68 ± 80.53^{a}	387.21 ± 19.91^{bc}	*
Lactate Dehydrogenase (IU/L)				
Fresh semen	638.00 ± 28.52^{e}	1031.71 ± 247.40^{d}	696.34 ± 36.78^{e}	*
Diluted semen	1146.71 ± 143.26^{e}	1339.15 ± 273.92^{de}	2123.59 ± 250.63^{c}	*
Post thaw semen	2362.59 ± 396.41^{bc}	3477.62 ± 494.30^{a}	3149.34 ± 408.34^{a}	*
Creatine Phosphokinase (IU/L)				
Fresh semen	226.685 ± 21.54^{d}	354.625 ± 93.74^{cd}	303.750 ± 47.66^{cd}	*
Diluted semen	381.625 ± 38.38^{cd}	507.032 ± 177.81^{bcd}	421.750 ± 77.61^{cd}	*
Post thaw semen	611.597 ± 119.40^{bc}	1090.782 ± 231.84^{a}	773.152 ± 118.89^{ab}	*
ϒ-Glutamyl Transferase (IU/L)				
Fresh semen	1298.75 ± 114.16^{c}	1498.62 ± 95.23^{c}	1381.09 ± 105.70^{c}	NS
Diluted semen	1586.78 ± 153.59^{c}	2140.18 ± 301.40^{c}	1886.21 ± 156.37^{c}	NS
Post thaw semen	4453.06 ± 204.79^{b}	6320.71 ± 1373.53^{a}	4823.18 ± 434.26^{b}	*
Testosterone ng/ml				
Testosterone	0.12 ± 0.02^{c}	0.92 ± 0.07^{a}	0.73 ± 0.06^{b}	**

^{a,b,c} Values within a row with no common superscript differed significantly (*P<0.05, **P<0.01)
NS - Nonsignificant

3.1. Aspartate transaminase

The differences in the mean values of AST between winter and summer and winter and monsoon was statically significant (p<0.05) and summer and monsoon remained non-significant. The differences in the values of diluted semen during winter and summer and summer and monsoon are statistically significant (p<0.05) in fresh semen. In post-thaw semen, significantly (p<0.05) highest values are recorded during summer and the lowest value during winter, but the difference between winter and monsoon is non-significant.

In the present study, the concentration of AST in the seminal plasma was highest during the summer season. The rise in AST may be due to leakage from spermatozoa. The concentration of AST has progressively increased from fresh to diluted to frozen semen. The best semen extender can boost up the sperm motility/viability and block the leakage of enzymes and other electrolytes from the sperm cell. The semen dilutor also plays an important role in releasing enzymes in the extracellular medium [14]. Rajoriya *et al.* [15] also reported a significant difference in AST activity in fresh semen between the winter and summer seasons of Tharparkar bulls, which is in accordance with the current study.

3.2. Alkaline phosphatase

The highest value of alkaline phosphatase enzyme was reported during summer and the lowest in fresh and diluted semen samples during the winter season. The differences in the values of ALP between winter and summer and summer and monsoon in fresh and diluted semen samples were statistically significant (p<0.05). However, in frozen/post-

thawed semen, higher ($p<0.05$) values were noticed during monsoon compared to winter and summer. The difference between summer and winter remained non-significant.

The values of ALP concentration in fresh, diluted and frozen semen during different seasons could not be compared as no work on seminal plasma alkaline phosphatase values in Holstein Friesian X Gir crossbred breeding bulls could be traced in the available literature. The higher value of ALP of fresh and diluted semen samples during summer in the present study agrees with Dhami and Kodagali [16] in buffalo. Seminal plasma phosphatase plays an important dephosphorylating role in sperm metabolism. The phosphatase enzyme in semen reflects the functional state of accessory sex glands and the metabolic activity of spermatozoa. Therefore, the estimation of enzymatic activities in seminal plasma reflects sperm membrane integrity and are helpful in differencing the reproductive biology of bulls of different breeds/ species.

3.3. Acid Phosphatase

Significantly ($p<0.05$) highest value of acid phosphatase enzyme was reported during the summer season in fresh, diluted and frozen semen samples. However, the difference between winter and monsoon remained non-significant. Khawskar *et al*. [17] also reported significant seasonal variation in seminal plasma enzyme profile in Surti buffalo bulls. The ACP concentration was higher in the summer season and lowest in the monsoon season. The finding of the present study that ACP concentration is higher in the summer season is in agreement with the present study. No reports are available in the literature in which a comparison has been made regarding Holstein-Friesian X Gir crossbred breeding bulls in seminal plasma acid phosphatase.

3.4. Lactate Dehydrogenase

Lactate Dehydrogenase concentration was significantly ($p<0.05$) higher during summer in fresh and post-thaw samples, whereas it was higher in diluted semen samples during monsoon. However, the lowest concentration was observed in fresh, diluted and frozen samples during winter. The concentration of LDH has drastically increased from fresh to diluted frozen semen samples in the present study. Rajoriya *et al.* [15] found a non-significant difference in LDH activity in Tharparkar bulls between the winter and summer season in fresh semen samples and post-thaw stage of cryopreservation, whereas, during the pre-freeze stage, there was a significant difference in LDH activity between summer and winter season.

3.5. Creatine Phosphokinase

The highest value of CPK enzyme was reported during summer and the lowest during the winter season in fresh, diluted and frozen semen samples. Creatine phosphokinase levels in human sperm are an objective biomarker of sperm maturity and fertilizing potential. CPK concentration in seminal plasma drastically increased from fresh to diluted to frozen semen samples. This could be due to the leakage of the enzyme due to freezing [14]. No reports are available in literature in which a comparison has been made regarding Holstein-Friesian X Gir crossbred breeding bulls with respect to CPK.

3.6. ϒ-Glutamyl Transferase

It is observed that ϒ-glutamyl transferase was non-significantly higher during summer and lower during the winter season in fresh and diluted semen, whereas significantly ($p<0.05$) higher in frozen semen samples and lowest in winter. GGT plays a major role in the glutathione system that is involved in the protection of spermatozoa against oxygen radicals [9]. The concentration of GGT in seminal plasma increased from fresh to diluted semen samples, while there was a drastic increase in GGT concentration from diluted to frozen semen samples. This could be due to the leakage of the enzyme due to the freezing of semen samples [14].

3.7. Testosterone concentration

The testosterone concentration was significantly ($p<0.05$) higher during summer, followed by monsoon and winter. This is in accordance with Javed *et al*. [18], who reported the influence of season on the seminal plasma testosterone in buffalo bulls.

4. Conclusions

It was concluded from the present study that seminal enzyme activities and testosterone showed significant differences between the season in Holstein Friesian crossbred breeding bulls in fresh, diluted and frozen semen samples. These animals are unable to withstand the change in environmental temperature; therefore, management of the breeding bulls of exotic inheritance should be undertaken to reduce the effect of heat stress and improve fertility.

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