B-5437
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RESEARCH ARTICLE Indian Journal of Animal Research

Semen Quality Correlation with Seminal Biochemistry and Enzymatic Profile of Barbari and Sirohi Bucks under Sub Tropical Environment

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10.18805/IJAR.B-5437

ABSTRACT

Background: The objective was to study the effect of seasonal variation on reproductive parameters, biochemical parameters and enzymes activity in relation to buck semen quality which would assist to produce good quality sperm doses for artificial insemination in goat farming.

Methods: Four ejaculates fortnightly were collected from adult breeding Sirohi and Barbari bucks during summer and winter season by using artificial vagina for the study. Average meteorological data were recorded during the study. Reproductive parameters (scrotal temperature and scrotal circumference) were recorded and fresh semen samples were used for evaluation of sperm attributes (semen volume, pH, sperm concentration, mass motility, individual activity and live-dead sperm count). The seminal plasma was separated by from semen for estimation of different biochemical parameters (Total protein, Albumin, Total cholesterol and Calcium) and seminal enzymes activity (ALT, AST, ALP and LDH).

Result: It was observed that sperm attributes and biochemical parameters were significantly ($p<0.05$) increased during winter as compared to summer season in both breed. No significant difference was observed for ALT in both breeds and seasons. Whereas, in summer AST activity was significantly ($p<0.05$) lower than winter in both the breeds. In summer season ALP and LDH activity were significantly ($p<0.05$) higher than winter in both the breeds. A positive correlation had observed with seminal biochemical parameters whereas a negative correlation with ALP and LDH. In conclusion, season influenced the semen quality in Barbari and Sirohi bucks and good quality semen produce during winter as compared to summer.

Key words: Biochemical parameter, Buck, Season, Semen quality.

INTRODUCTION

Goats have enormous promise as the “Future animal” for rural development in the face of shifting agro-climatic conditions and depleted resources. There are several factors that affect the semen quality and quantity as well as fertility in animals. Although goats had experienced many kinds of stresses, environmental stress is the one that needs to concern us the most. Changes in the climate affect the productivity of goat and causes economic losses to farmers (Kumar *et al.,* 2017). Seasonal variation influences the reproductive activity by affecting testicular weight and size, sperm generation, testicular secretion, mating activity and ultimately the fertility (Zarazaga *et al*., 2009). Over 40% of inseminated sires are impacted by seasonal variation in terms of proteins, seminal enzymes and oxidative status. In summer buck scrotal circumference, diameter, biochemical parameters and semen quality were lower than in the winter (Mohamed *et al*., 2023).

In males, the testes and accessory sex glands produce and exude seminal plasma, which plays an important role in the development of sperm motility and freezing capacity. Seminal plasma protein low concentration is indicator of poor semen quality. Season had a major impact on the protein level in seminal plasma. A positive relationship was described between scrotal circumference, sperm motility, sperm concentration and level of total protein in buck seminal plasma (Arrebola and Abecia, 2017). Spermatozoa require seminal plasma albumin to function properly. Seminal plasma albumin serves as a depot for cholesterol, which is released from the sperm membrane during capacitation. It stimulates sperm motility, causes acrosome reactions and protects the membrane from lipid peroxidation. Umar *et al*. (2018) studied the correlation between the biochemical constituents of seminal plasma and semen quality.

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How to cite this article: Khan, A., Mandal, S., Gattani, A., Nath, S., Jain, A.K., Jesse, D.D., Dhurvey, M., Mishra, A. and Patel, P. (2024). Semen Quality Correlation with Seminal Biochemistry and Enzymatic Profile of Barbari and Sirohi Bucks under Sub Tropical Environment. Indian Journal of Animal Research. 1-7 doi:10.18805/IJAR.B-5437

Submitted: 01-07-2024 **Accepted:** 22-10-2024 **Online:**06-12-2024

The release of the intracellular enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) into extracellular medium signifies sperm cell damage. Transaminases are mainly found in the spermatozoa's mid section. However, lactate dehydrogenase (LDH) is found in the spermatozoa's mitochondria and cytosol. These enzymes can be thought of as good indicators of the quality of the semen because they measure the spermatozoa's plasma membrane stability (Juma and Kassab, 2009). Seminal LDH and ALP, indicates the metabolic status of the spermatozoa (Juyena and Stelletta, 2012).

The present study was conducted to investigate the correlation between sperm attributes with reproductive parameters, seminal plasma biochemical parameters and enzyme activity varied throughout the summer and winter season in Barbari and Sirohi bucks. A better knowledge on the association and correlation between the above parameters in the seminal plasma and spermatozoa during the semen collection would assist to prepare the quality sperm doses for artificial insemination to meet the demand of buck semen for goat farming.

MATERIALS AND METHODS

The experiment followed the criteria of the Institutional Animal Ethics Committee (IAEC). The meteorological data records were taken from Meteorological Centre, Anand Nagar, Adhartal, Jabalpur (M.P.). Twelve adult Sirohi and Barbari breeding bucks of 1.5-2 years age were used in study. All breeding bucks were managed under organized Amanala Goat Farm, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.) India. Jabalpur is located at latitude 23°10′N and longitude 79°56′E at an altitude of 411 meters in central India. It has a humid subtropical climate, typical of North-Central India with a seasonal variation in temperature ranging from 10.2°C to 41.9°C. The study was conducted in two season *viz.* summer (May-June) and early winter (November-December) using which temperature, sunshine hours and relative humidity was recorded for 50 days before the date of collection.

Scrotal circumference (cm) was measured using flexible commercially available measuring tape. Scrotal temperature (°C) was measured by thermo scanner. Four ejaculates fortnightly were collected from twelve adult breeding bucks of Sirohi and Barbari breed during summer and winter season by using artificial vagina. Fresh semen samples were macroscopically and microscopically assessed for qualitative characteristics during summer and winter. Volume of semen was measured in a graduated semen collecting tube. A pH meter was used to estimate the pH of the semen. Sperm concentration was estimated by using Neubauer haemocytometer. For progressive motility (Hafez and Hafez, 2000) a drop of diluted semen (1:200 dilutions) in isotonic saline solution was placed on a pre-warmed, clean, grease-free slide. A cover slip was placed over it to ensure that the drop spread uniformly. Three fields were randomly inspected using the high power (40X) objective. Live sperm count was estimated by eosin-nigrosin staining method by examining the stained smears under microscope at high power objective (Deori *et al.*, 2018).

For biochemical analysis in summer and winter season semen samples, were centrifuged at 3000 RPM for 10 minutes and seminal plasma was kept at -20°C for further analysis. Seminal plasma total protein (Biuret method), Albumin (BCG dye method), total cholesterol and Calcium were estimated by using commercial kits (ERBA) as per manufacture's instruction. The seminal plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP) and lactate dehydrogenase (LDH) activity was estimated as per commercial kits (ERBA) by using semi autoanalyzer. The data were analysed with the help of SPSS software using repetitive measure ANOVA and obtained data from various experiments were provided as Mean±SE. A difference of $p<0.05$ was considered statistically significant.

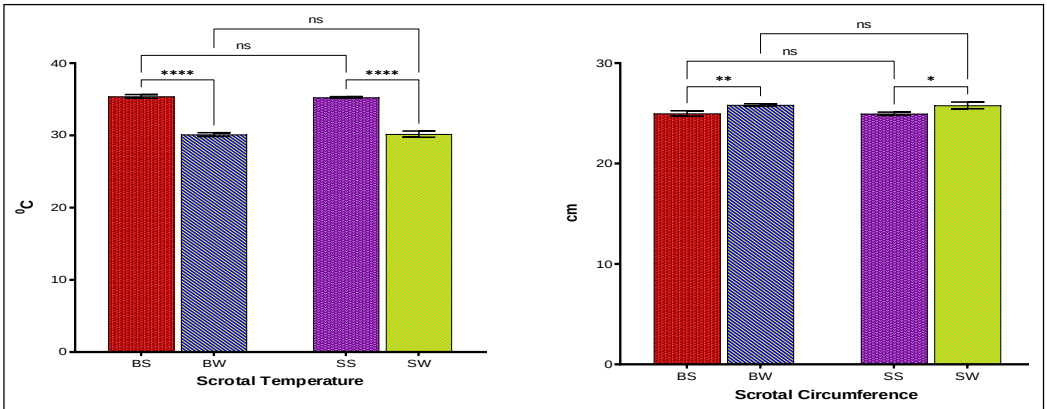
RESULTS AND DISCUSSION

The average meteorological variables were recorded at the experimental farm station during summer and winter season. During experimental period high mean temperature humidity index (THI) value was recorded during summer than winter season. Mean relative humidity in percentage recorded during summer and winter season were 41.23±1.66 and 78.95±1.23 respectively. Average temperature humidity index (THI) in percentage recorded during experimental period was 80.84±0.21 and 67.74±0.42 in summer and winter season, respectively.

In higher value of ambient temperature, reduced sperm quality and increased scrotal temperature was observed. A significantly ($p<0.05$) higher scrotal temperature (Fig 1A) was recorded in summer as compared to winter season, whereas there was a no significant effect of breed observed for scrotal temperature. The higher scrotal temperature during summer season might be due to metabolic heat production, altered scrotal circulation, environmental temperature and less scrotal sweat production (Salles *et al.*, 2020). A significantly ($p<0.05$) higher scrotal circumference (Fig 1B) was recorded in winter as compared to summer season, for both breeds. Elevation in temperature humidity index (THI) and day time length during summer was associated with decreased values of testicular dimension in buck *i.e.* scrotal circumference as compared to winter (Mohamed *et al.*, 2023). The body weight and testicular parameters were positively correlated with semen volume and negatively correlated with sperm concentration in all the breeds (Khan *et al.*, 2022) Increase buck testicular dimensions during winter could be contributed to the high testosterone concentration during the winter season which increases spermatogenesis.

Bucks responded differently during summer and winter season because of environmental stress during summer. The mean value of semen volume, pH, sperm concentration, mass and individual motility, live sperm

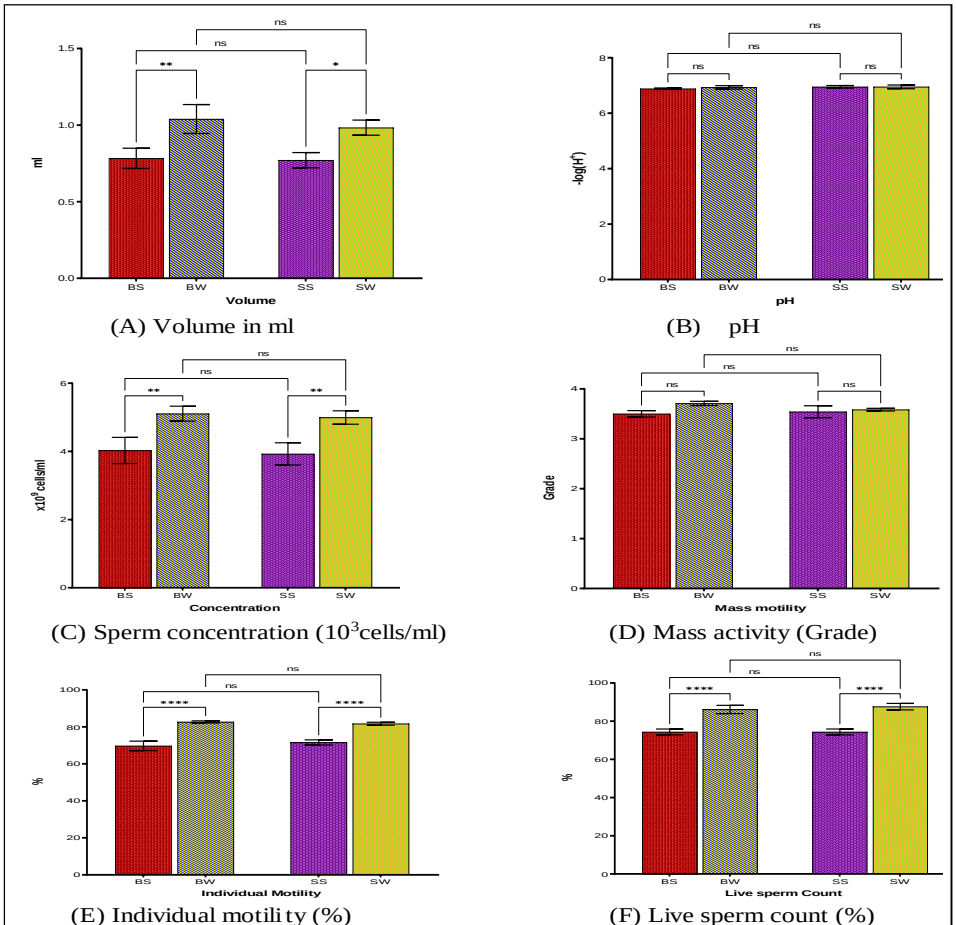
percentage in Barbari and Sirohi buck has been represented in (Fig 2). Semen volume (Fig 2 A) was significantly ($p<0.05$) higher in winter as compared to summer in both the breeds. In winter semen volume is increased mainly due to quantity of fluids secreted by the accessory sex glands and the epididymis which are



(A) Scrotal Temperature in ^{0}C (B) Scrotal Circumference in cm

(A) Scrotal Temperature (°C) (B) Scrotal Circumference (cm) of Barbari and Sirohi during summer and winter season (BS-Barbari Summer, BW- Barbari Winter, SS-Sirohi Summer, SW-Sirohi Winter).

Fig 1: (Mean±SE) Reproductive parameter.



(E) Individual motility (%) (F) Live sperm count (%)

(A) Volume (ml) (B) pH (C) Sperm concentration (10^3 cells/ml) (D) Mass activity (Grade) (E) Individual motility (%) (F) Live sperm count (%) of Barbari and Sirohi during summer and winter season (BS-Barbari Summer, BW- Barbari Winter, SS-Sirohi Summer, SW-Sirohi Winter).

Fig 2: (Mean±SE) oxidative parameters.

androgen dependent (Leboeuf *et al*., 2000). No significant difference was observed between the breed and season for semen pH parameter (Fig 2B). The amount of epididymal secretions produced was adequate to maintain the pH at a required and optimal level even in the summer. Sperm counts were significantly ($p<0.05$) higher in winter than summer in both the breeds whereas no significant difference was observed in between the breeds. Sperm concentration (Fig 2C) in present study was higher reported in bucks during different seasons by Qureshi *et al*. (2013), whereas it was lower than the values reported by Kulaksiz *et al*. (2019). The higher sperm concentration during winter season might be due to increased buck's testicular dimensions and high testosterone concentration during the winter season which increases spermatogenesis. In this study the observed mass motility (Fig 2D) of Barbari and Sirohi bucks showed non-significantly higher values in winter than summer. Individual motility (Fig 2E) was significantly ($p<0.05$) higher during winter than summer in both the breeds. Sperm motility is low during summer season as compared to winter might be due to high THI which reduces the accessory sex gland secretions results in decreased sperm quality as well as motility. Live percentage (Fig 2F) of sperm were significantly ($p<0.05$) higher during winter than summer in both the breeds. Ranjan *et al*., 2020 also reported a significantly ($p<0.05$) lower live sperm percentage during summer than winter in Barbari buck semen. High ambient temperatures have a negative effect on male fertility, interfere with spermatogenesis and sperm production and increase the percentage of dead sperm.

The mean value of total protein, albumin concentration, total cholesterol and calcium concentration in Barbari and Sirohi breed has been represented in Table 1. A non-significant ($p>0.05$) difference was observed for the seminal total protein concentration, albumin concentration, total cholesterol and calcium in two breeds, whereas the effect of the season on the parameters were significant ($p<0.05$) higher during winter as compared to summer in both the breeds. Total protein concentration was significantly higher in winter as compared to summer which was contradicted to the finding of Dhilon *et al.* (2020). However, it was similar with findings of Arrebola and Abecia (2017) who found winter ejaculates had the highest protein concentrations compared to summer. Decrease protein contents of seminal fluid in summer season relates to the reduced semen quality. Seminal plasma albumin plays an important role as a reservoir for cholesterol, which is removed from the sperm membrane during capacitation. It stimulates sperm motility, inhibits acrosome reaction and protects the membrane from lipid peroxidation. Albumin sequesters the free radicals thereby protecting the sperms against lipid peroxidation and oxidative stress. The higher concentration of albumin during winter season might be due to less oxidative stress status. Higher ambient temperature leads to decrease in total cholesterol concentration, which may be related to increased use of fatty acids for energy production in animals because of lower glucose concentration in summer. Calcium is involved in initiating many reactions including the motility of spermatozoa which is higher during winter season in our findings similar to Dias *et al*. (2017).

The important enzymes for metabolism *viz.* transaminases, lactate dehydrogenase and alkaline phosphatase were studied in the present study and mean value of findings has been represented in Table 1. The transaminase in the semen is concern with the oxidative

Table 1: Seminal plasma biochemical parameters of barbari and sirohi during summer and winter season (Mean±SE).

Parameter	Season	Breed	
		Barbari	Sirohi
Total protein (g/dl)	Summer	4.20^{a}±0.14	4.11^{a} ±0.10
	Winter	4.82^{b}±0.21	4.65^{b}±0.16
Albumin (g/dl)	Summer	1.62^{a}±0.11	1.60^{a}±0.09
	Winter	2.08^{b}±0.11	2.20^{b}±0.11
Total cholesterol (mg/dl)	Summer	47.43^{a}±1.12	46.96^{a}±2.56
	Winter	68.95^{b}±1.68	67.52^{b}±1.13
Calcium (mg/dl)	Summer	8.42^{a}±0.28	8.47^{a}±0.27
	Winter	9.44^{b}±0.16	9.61^{b}±0.14
ALT/SGPT (IU/L)	Summer	20.17^{b}±1.21	18.29^{b}±1.29
	Winter	19.33^{a}±0.91	17.78^{a}±1.16
AST/SGOT (IU/L)	Summer	101.80^{a}±2.05	98.90^{a}±4.78
	Winter	119.40^{b}±2.96	121.20^{b}±3.41
ALP (IU/L)	Summer	54.80^{b}±2.21	53.89^{b}±1.02
	Winter	49.30^{a}±0.91	48.56^{a}±0.96
LDH (IU/L)	Summer	222.50^{b}±4.79	227.80^{b}±8.89
	Winter	216.30^{a} ±5.96	217.60^{a}±5.63

Note: Mean superscripted with different small letters column wise differ significantly ($P\leq0.05$) from each other.

metabolic pathways and provides energy substrate to the sperm cells. No significant difference was observed for the mean value of alanine transaminase (ALT) in both breeds and seasons. In summer aspartate transaminase concentration was significantly ($p<0.05$) lower than winter in both the breeds. The transaminases activities (AST and ALT) in semen are a good indicator of semen quality because it measures sperm membrane stability (Corteel, 1980). The increase of AST activity in seminal plasma as an index of degree of sperm damage leading to increase sperm membrane permeability to AST (Pace and Garaham, 1970). Our present research was also similar with the finding of Juma and Kassab (2009). In summer alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activity were significantly ($p<0.05$) higher than winter in both the breeds. Alkaline phosphates (ALP) are known to be involved in several cellular activity in the male reproductive system, our result reflect a marked increase in summer than winter in both the breeds. The present study agreed with other researchers report (Juma and Kassab, 2009). The increase in ALP activity in summer may be due to increase secretion of adrenocorticotrophic hormone (ACTH)

Table 2: Pearson's correlation coefficients between sperm attributes, reproductive and biochemical parameters of Barbari during summer and winter season (Mean±SE).

	IM	MA	Vol	Conc	LSC	SC	ST	TP	Alb	TC	Cal	AST	ALP	LDH
IM	1.00	0.19	0.21	0.51	0.55	-0.53	-0.78	0.47	0.38	0.78	0.25	0.72	-0.46	-0.52
MA		1.00	0.06	0.41	0.18	-0.09	-0.18	0.08	-0.02	0.25	0.13	0.07	-0.18	-0.19
Volume			1.00	0.05	0.50	-0.12	-0.43	0.13	0.24	0.54	0.17	0.38	-0.09	-0.11
Conc.				1.00	0.31	-0.54	-0.41	0.32	0.34	0.49	0.08	0.36	-0.43	-0.21
LSC					1.00	-0.32	-0.69	0.41	0.41	0.77	0.45	0.59	-0.48	-0.21
SC						1.00	0.63	-0.47	-0.36	-0.55	-0.22	-0.57	0.45	0.23
ST							1.00	-0.45	-0.44	-0.87	-0.46	-0.82	0.57	0.43
TP								1.00	0.06	0.45	0.05	0.29	-0.24	0.07
Albumin									1.00	0.49	0.16	0.37	-0.49	-0.26
TC										1.00	0.42	0.74	-0.49	-0.44
Cal											1.00	0.34	-0.01	-0.08
AST												1.00	-0.55	-0.56
ALP													1.00	0.51
LDH														1.00

IM- Individual motility, MA- Mass activity, Vol- Volume, Conc- Concentration, LSC- Live sperm count, ST- Scrotal temperature, TP- Total protein, Alb- Albumin, TC- Total cholesterol, Cal- Calcium, AST- Aspartate transaminase, ALP- Alkaline phosphatise, LDH- Lactate dehydrogenase.

Table 3: Pearson's correlation coefficients between sperm attributes, reproductive and biochemical parameters of Sirohi during summer and winter season (Mean±SE).

	IM	MA	Vol	Conc	LSC	SC	ST	TP	Alb	TC	Cal	AST	ALP	LDH
IM	1.00	0.29	0.36	0.24	0.79	-0.62	-0.82	0.45	0.52	0.84	0.27	0.56	-0.65	-0.39
MA		1.00	0.36	0.07	0.05	-0.11	-0.18	0.16	-0.03	0.04	0.14	0.16	-0.36	-0.08
Volume			1.00	0.20	0.32	-0.34	-0.27	0.07	0.29	0.29	0.06	0.03	-0.04	-0.19
Conc.				1.00	0.42	-0.31	-0.44	0.21	0.36	0.34	0.44	0.36	-0.36	-0.23
LSC					1.00	-0.57	-0.76	0.35	0.39	0.84	0.36	0.56	-0.56	-0.34
SC						1.00	0.77	-0.33	-0.43	-0.64	-0.12	-0.60	0.51	0.51
ST							1.00	-0.39	-0.44	-0.81	-0.38	-0.72	0.75	0.47
TP								1.00	0.20	0.40	0.39	0.26	-0.47	0.26
Albumin									1.00	0.39	0.11	0.35	-0.34	-0.36
TC										1.00	0.36	0.71	-0.51	-0.35
Cal											1.00	0.25	-0.35	-0.08
AST												1.00	-0.52	-0.41
ALP													1.00	0.47
LDH														1.00

IM- Individual motility, MA- Mass activity, Vol- Volume, Conc- Concentration, LSC- Live sperm count, ST- Scrotal temperature, TP- Total protein, Alb- Albumin, TC- Total cholesterol, Cal- Calcium, AST- Aspartate transaminase, ALP- Alkaline phosphatise, LDH- Lactate dehydrogenase.

due to environmental stress (Litwack, 1972). Farshad *et al.* (2012) also reported lower LDH activity during winter season in comparison to summer season. This enzyme can also be thought of as good indicator of the quality of the semen because they measure the spermatozoa's plasma membrane stability. LDH is an intracellular enzyme and elevated levels in the seminal fluid may indicate the poor integrity of the sperm plasma membrane, making it a good indicator for this purpose.

As presented in Table 2 and Table 3, sperm motility had significant positive correlation with other semen attributes *i.e.* semen volume, semen concentration and mass activity whereas negative correlation with reproductive parameter (Scrotal circumference and scrotal temperature). A positive correlation had observed with seminal biochemical parameters *i.e.* total protein, albumin total cholesterol, calcium whereas negative correlation with ALP and LDH. Previous studies also reported the same results in agreement with the present findings Dhara *et al.* (2022). Umar *et al*. (2017) observed significant correlation of biochemical and enzymatic parameters were also positively or negatively correlated with all buck semen attributes of both breed.

CONCLUSION

Semen attributes of bucks of both breeds affected by climatic condition and better quality semen produces during winter season as compared to summer. High temperature humidity index (THI) during summer appears to impair reproductive parameters, seminal biochemical parameters and enzymatic activity potentially compromising semen quality. These findings suggest that early winter is the best season typical of North-Central India humid subtropical climate, for maintenance of sperm viability, modulates sperm function and preparation of quality sperm doses for artificial insemination to meet the demand of buck semen for goat farming.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, College of Veterinary Science and A.H., Jabalpur (M.P.) for his support given to the present study.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflicts of interest

The authors declare no conflicts of interest.

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