



Influence of seasons and THI on reproductive performance and in vitro assay of semen of Barbari buck under tropical conditions

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ABSTRACT

Temperature fluctuations due to climate change, have important impact on sexual behaviour and seminal attributes. The present study aimed to evaluate the effect of summer and monsoon seasons on the sexual behavior pattern (RT: reaction time and SA: sexual aggressiveness) and seminal characteristics of Barbari bucks (*Capra hircus*). The semen samples were harvested and evaluated at weekly intervals during summer and monsoon season using standardized methods from Barbari buck (n=6; age 1.5 to 2.5 yrs.). The RT varied considerably from the hot, dry condition of summer to the humid, wet weather of the monsoon, whereas no significant seasonal variation was observed in the SA of Barbary bucks. Significant (P<0.05) improvement was noted in RT (seconds) and the semen-related parameters during monsoon season as compared to summer season. The temperature-humidity index (THI) and dry bulb temperature did not differ significantly among the study seasons. Significantly higher relative humidity (78.15±0.60 vs 61.17±1.46; P<0.05) and RT (3.25±0.10 vs 2.80±0.11; P<0.05) were observed during the monsoon season as compared to the summer season. In conclusion, Barbari bucks had better seminal quality parameters and reaction time in monsoon seasons than in summer seasons.

Keywords: Barbari bucks, Seasons, Seminal quality parameters, Sexual behavior

Tropical climate has an important bearing in fulfilling the increasing demand for milk, meat, and other animal products across the globe (Saba, 2024). Unlike other domestic animals, goats have better resilience against varied climatic conditions (Ramachandran and Sejian, 2022). Like other tropical goat breeds, the Barbari goat (*Capra hircus*), a popular famous dual purpose, for both milk and meat purpose, and heat-tolerant breed. It has high reproductive efficiency and production performance is affected to high extreme weather conditions. However, throughout the year reproductive efficiency of these bucks exhibited considerable changes affected by environmental conditions, particularly relative humidity and environmental temperature. The prolong aggregation of metrological constituents and its fluctuations, which define weather during brief intervals, is emphasized (Krol, 2025) as stress. Stress is defined as the compromise of 'minimum complete comfort' in life due to surrounding

biotic and abiotic variables that induce discomfort in animals (Sejian *et al.* 2015). The Heat Stress Index or THI, utilized to assess human comfort and thermal stress (Thom, 1959), remains the predominant metric for evaluating domestic animal conditions, based solely on temperature and humidity (Pinto *et al.* 2020).

Different THIs reflect the combined function of relative humidity and environmental temperature on production, reproduction as well as animal stress (Kumar *et al.* 2018). Over the past few years, in response to rising trend of global climate disruption and its significant effects on goat husbandry. Scientists have heightened their efforts to comprehend how climatic variables, such as Heat Stress Index or THI stress induced reproductive dysfunction of male Barbari goat (Marino *et al.* 2016), like THI, impact the bucks reproductive physiology. Elevated THI values are linked to poor quality of semen across different goat breeds (Ingraham *et al.* 1974), including Barbari bucks. These negative consequences can result in decreased reproductive efficiency in both bucks as well as does, ultimately impairing overall fertility outcomes and presenting significant challenges to viable goat husbandry operations (Danso *et al.* 2024). Regardless of the expanding corpus of study explaining the connection the correlation between reproductive characteristics and THI in domestic animals (Manyike *et al.* 2025), there remains a scarcity in depth analyses, specifically focusing on the Indian

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tropical climate. In goat herd development projects, buck fecundity is crucial as it significantly influences the herd's reproductive capability along with genetic enhancement, while the reproductive periodicity substantially affects the overall semen quality (Bodu *et al.* 2025).

Considering their commercial significance and insecurity to adverse environmental factors, it is essential to evaluate the effect of THI on fertility related parameters (Pardo *et al.* 2022), namely semen related parameters and sexual desire of male goats under tropical climate in India.

MATERIALS AND METHODS

Location of study: The study was conducted at Deep Frozen Semen (DFS) Laboratory and Department of Veterinary Physiology and Biochemistry, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (U.P.) India.

Ethical approval: The experiment was approved by the institutional animal ethics committee of the college vide letter no. P-3/2025.

Experimental animals: Six experimental bucks (Barbari breed, aged 1.5-2.5 years) were reared under similar feeding and managerial conditions at a DFS Lab., ANDUAT, Kumarganj-224229, Ayodhya (UP), India. All the bucks had previously been trained to ejaculate into the AV while utilizing a dummy buck.

Environmental variables: The environmental data was obtained from the Department of Agricultural Meteorology, ANDUAT Kumarganj-224229, Ayodhya, India, located at geographical coordinates are Latitude: 26.47°N; Longitude: 82.12°E and 113.00 meters above sea level. The analysis encompassed average ambient temperature or Tdb and air saturation (RH) for the entire research duration. The values were documented every day at 07:00 a.m. during semen collection, and the mean monthly values from the beginning to the end of the research.

THI calculation: To assess the impact of the Heat Stress Index or THI on fertility data, the ambient temperature or Tdb and air saturation (RH) for each month were obtained from the Department of Agricultural Metrology, ANDUAT Kumarganj, Ayodhya. The THI was computed according to the method provided by Ranjan *et al.* (2020) and Kumar *et al.* (2018) with the mathematical equation:

$$\text{THI} = \text{TdB} - [0.55 - (0.55 \times \text{RH}/100)] \times (\text{TdB} - 58)$$

**THI-Temperature Humidity Index; DBT/Tdb, (°F)-Dry Bulb Temperature in degrees Fahrenheit; RH- Relative Humidity (Kumar, *et al.* 2018; Ranjan *et al.* 2020). The THI was categorized in group of four (Messeri *et al.* 2023).

The acquired data were evaluated to assess the impact of THI on mating behavior and semen parameters.

Sexual behavior: The sexual behavior of the buck was consistently observed through CCTV without interfering with the buck's behavior. The frequency of sexual interactions involving other bucks (sniffs, anogenital contact, flehmen response, and nudging) (Shackleton & Shank, 1984) and covering behaviors (cover attempts,

cover with and without ejaculation) were documented before semen ejaculation and during semen collection according to the subsequent criteria:

Sexual aggressiveness (SA): The male goat conduct throughout their address to another buck and a dummy doe was visually evaluated and categorized. a) Assertive: Highly enthusiastic to engage the female goat with utmost intensity. b) Energetic: Tackled the female goat with diminished stamina and hostility. b) Slow: Advanced with a lackluster demeanor and required an extended duration to ascend. d) Shy: Displayed limited arousal and was hesitant to engage in mounting.

Reaction time (RT): The RT refers to the duration taken by buck to mount after being presented with a dummy doe or another buck in this study.

Table 1. Categorization & Mating record of Reaction Time and Sexual Aggressiveness of bucks

S.No.	Reaction Time (sec.)	Score	Sexual Aggressiveness	Score
1.	<20	4	Assertive	4
2.	21-40	3	Energetic	3
3.	41-60	2	Slow	2
4.	61-80	1	Shy	1

(Ahmed, 1997; Barik *et al.* 2009; Kerketta *et al.* 2014; Shastri *et al.* 2025)

Semen collection and processing: The of semen ejaculate was collected weekly using a standardized AV method. Two ejaculates were collected from each buck, with a brief interval of rest between the two procedures. The ejaculates were conditioned in a water bath for stabilization during the study period. Ejaculates exhibiting significant variation in volume, pH, sperm concentration, mass, individual progressive motility, and abnormal color patterns were excluded from the study. The remaining high-quality ejaculates were then subjected to microscopic analysis and processed for further investigation. Following this, initial assessments of the 144 ejaculates from six Barbari bucks across two seasons (summer and monsoon) involved pooling two consecutive ejaculates from the same buck. Semen exhibiting mass activity of 4 or higher and progressive motility exceeding 70% was subsequently prepared for freezing.

Sperm activity: The mass motility of the semen sample was assessed by placing a small drop of freshly collected neat semen on a clean, pre-warmed glass slide at 37°C. The sample was examined without a cover slip under the 100X power magnification of a phase contrast microscope. Mass activity was assessed using a scale from 0 to 5, determined by the presence of waves and swirls.

Sperm concentration: The sperm concentration ($\times 10^6$ /millions/mL) and sperm per ejaculate (SPE) in a neat semen sample were calculated using the hemocytometer method. The sperm count was observed by a trained technician with a fluid used to maintain the quality of a semen sample

for a period a time (Centola, 2005). The uniformity of the sample was ensured by mixing the manual semen assessment (MSA) and also during the process of loading the hemocytometer.

Sperm viability or livability: The assessment of sperm viability involved counting both viable and non-viable specimens by standard procedures, and morphological analysis of a 200-sperm cell sample using a Phase Contrast Microscope. A careful documentation of viable and non-viable sperm counts, which were subsequently calculated as a percentage. The stained spermatozoa exhibited a pink hue (eosinophilic) and were confirmed as non-viable, while the unstained ones, observed against a darkened Nigrosin, were recorded as viable (Hancock, 1951; Ranjan *et al.* 2022; Kumar *et al.* 2024; Shastri *et al.* 2025).

Abnormalities in sperm: The investigation into morphological sperm abnormalities was conducted utilizing the eosin-nigrosin staining technique. The semen was aliquoted and spread in a thin film on grease-free slide. A minimum of 200 spermatozoa was counted from multiple microscopic fields of view on the slide, and every abnormality was recorded for each smear at a magnification of 100X using an oil immersion lens to assess the incidence of various sperm abnormalities. Different types of sperm abnormalities have been categorized into head, mid-piece, and tail abnormalities (Youngquist & Threlfall, 2006; Menon *et al.* 2011; Bhoi *et al.* 2023). The ratio of each was determined and represented as a percentage.

Acrosomal integrity: The Giemsa staining technique was employed to investigate acrosomal integrity (Watson, 1975). A thin smear from each ejaculate was meticulously prepared onto a pre-clean, microscopic dry slide and subsequently permitted to air dry. Following the drying process, the semen smear was promptly fixed in Hancock's fixative for a duration of 15 minutes. The fixed semen smear was subsequently rinsed in slow-running tap water for a duration of 20 minutes. Following the application of the Giemsa working solution, a total of 200 spermatozoa were analyzed in each smear using the oil immersion objective of a compound microscope at a magnification of 1000X. Acrosomal integrity was assessed as the percentage of intact acrosomes.

Hypo-Osmotic Swelling (HOS) Examination: Cell membrane stability of spermatozoa was assessed (Jayendran *et al.* 1984) using a HOS solution (osmotic pressure 100 mOsmol/kg) prepared following the procedure of Prasad *et al.* (1999). The temperature of the incubator was held at 37 °C for 5 minutes pre-test. Following incubation, a droplet of the mixture was analyzed using a high-power phase-contrast microscope (40X) to observe various swelling patterns (Prasad *et al.* 1999). A total of two hundred spermatozoa were evaluated for their swelling capability in HOS, with sperm cells exhibiting patterns B, C, and D were deemed to possess an intact plasma membrane.

Sperm penetration distance (SPD): In Vitro Fertility Assay Polyacrylamide Gel Penetration Test (PAGPT) was conducted to assess SPD. A capillary tube filled with gel was

positioned vertically in 50 µL of semen contained within a microcentrifuge tube, and the samples were treated at 37 °C for 30 minutes. Observations of spermatozoa in the gel were conducted at magnifications of 10X and 40X using a bright-field microscope. The peak point was identified as the maximum distance traveled by the most progressive spermatozoa. The spermatozoa count was conducted at a distance of less than 0.5 cm from the peak point.

Statistical analysis: The complete sets of findings relevant to the ongoing investigations were framed as mean \pm SEM. The data underwent statistical analysis through one-way ANOVA utilizing OPSTAT (Sheoran *et al.* 1998) and Graph Pad Prism software (Swift, 1997) to perform a test of significance between the groups. The difference at $P \leq 0.05$ was statistically significant.

RESULTS AND DISCUSSION

This investigation presented the data collected during the summer and monsoon season, where the overall mean values for semen volume, concentration, sperm in one ejaculate or spermatozoon count, individual progressive motility, sperm livability, acrosomal integrity, sperm abnormalities, plasma membrane integrity, and sperm penetration distance were recorded (Fig 1). The climatic variables, including the average ambient temperature or Tdb (°C), air saturation (RH) (%), and Heat stress index or THI, observed throughout the experimental period are presented in Table 2.

Reproductive performance: The present research examined the sexual behavior of experimental bucks (Table 1) and revealed a significantly higher reaction time ($P < 0.05$) during the monsoon as compared to the summer seasons. Although there was a higher level of sexual aggressiveness observed during the monsoon season, this observed difference was not statistically significant ($P < 0.05$) when compared to the summer months. Observed libido score (%) was notably elevated ($P < 0.05$) in Barbari bucks showing significant difference from that observed earlier in the summer months observed in Mehsana bucks (ALI, 2022). According to the findings of Qureshi *et al.* (2013), there was a significant reduction in libido scores during the summer months in dairy goats ($P < 0.05$). The reproductive performance and RT of Deoni bulls also did not reach statistically significance in winter season,

Table 2. Environmental variables with THI of dry hot (summer) and Monsoon (rainy) seasons

S. No.	Environmental variable	Dry hot summer	Monsoon
1.	THI	85.53 \pm 1.15 ^a	85.86 \pm 0.52 ^a
2.	Dry bulb temperature (°C)	33.38 \pm 0.80 ^a	30.18 \pm 0.35 ^a
3.	Relative humidity (%)	61.17 \pm 1.46 ^a	78.15 \pm 0.60 ^b
4.	Reaction Time (Score)	2.806 \pm 0.11 ^a	3.250 \pm 0.10 ^b
5.	Sexual Aggressiveness (Score)	2.861 \pm 0.09 ^a	3.028 \pm 0.10 ^a

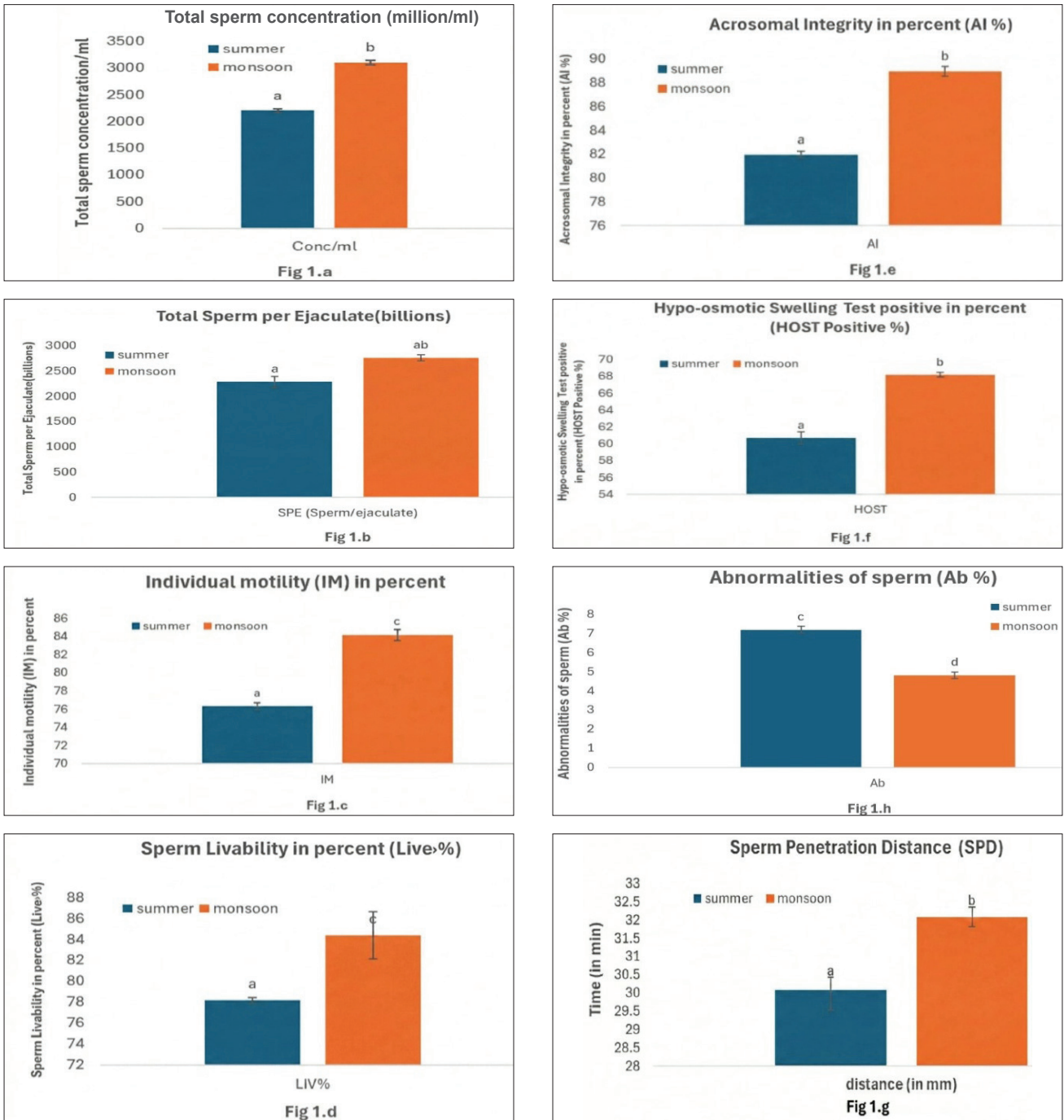


Fig. 1a, b, c, d e, f, & g. Impact of seasonal variation on semen parameters of Barbari male goat. Vol: volume (ml), Ab: Abnormality %. Sperm concentration (million/mL), SPE: sperm per ejaculate. IM: individual motility % LIV%: livability %, AI: acrosomal integrity %, HOST: hypo osmotic swelling test % , and SPD: sperm penetration distance (mm/20 minutes).

subsequently the monsoon and summer seasons, as reported by Kumar *et al.* (2024).

Seasonal changes in semen quality and sperm function:

The monsoon and summer seasons exhibit a noteworthy correlation among all six Barbari bucks. The correlation coefficient was determined to be significant and positive in relation to volume, livability, sperm per ejaculate, abnormality, and acrosomal integrity. Significant negative correlations were observed in concentration, individual motility, plasma membrane integrity, and sperm penetration

tests across both seasons (Fig.1). The identification of these environmental strains and seasonal variations in the reproductive efficiency of bucks might facilitate improved management practices for male and semen specimens utilized throughout various artificial selections. The Barbari goat exhibits an exceptional ability to regulate its body temperature, demonstrating adaptation to the challenging climatic conditions prevalent in Uttar Pradesh (Sejian *et al.* 2021). The authors noted that, although, other goat breeds in Uttar Pradesh, including Jamunapari and its crossbreeds,

were successfully propagated, persisted, and reproduced, albeit not to the same extent as the Barbari goat (Srivastava *et al.* 2021; Das *et al.* 2025).

Correlation analysis: Analysis of correlation indicated that the parameters of semen quality were interrelated ($P < 0.05$) during the summer season in Barbari buck goats. The volume, individual motility, livability, plasma membrane integrity, and sperm penetration test exhibited positive correlations ($P < 0.05$) among themselves. Conversely, these semen quality parameters demonstrated a significant inverse relationship with sperm concentration and acrosomal integrity ($P < 0.05$) during the summer seasons (Tab. 3b and Fig. 1a, b, c, d e, f, & g). The volume, sperm per ejaculate, livability, and abnormality exhibited a significant upward trend between the variables. In contrast, these semen quality parameters showed a significant inverse correlation ($P < 0.05$) with seminogram, individual motility, acrosomal and plasma membrane integrity, as well as the sperm penetration test during the monsoon seasons (Table 3a).

All these parameters are significantly correlated (≤ 0.05) with each other. Vol: volume (ml), Ab: Abnormality %. Conc (million/mL), SPE: sperm per ejaculate. IM: individual motility %, LIV%: livability %, AI: acrosomal integrity %, HOST: hypo osmotic swelling test %, and SPD: sperm penetration distance. Dry summer season: March to June.

The study indicated that in the summer season, there was

an inverse association ($P < 0.05$) with Tdb and temperature humidity index with RT, while relative humidity showed a positive correlation (Fig. 2 and Table. 4b). The analysis regarding SA in relation to relative humidity revealed a positive correlation ($P < 0.05$). Other parameters such as dry bulb temperature and temperature humidity index exhibited

Table 4a. Correlation with the reproductive performance and climatic variables of Barbari goat buck in monsoon seasons

	RT	SA	Tdb	RH	THI
RT	1				
SA	-0.01	1			
Tdb	-0.33	-0.51	1		
RH	0.43	0.29	-0.43	1	
THI	-0.27	0.49	0.98	-0.28	1

Table 4b. Correlation with the reproductive performance and climatic variables of Barbari goat buck in dry summer seasons

	RT	SA	Tdb	RH	THI
RT	1				
SA	-0.51	1			
Tdb	0.92	-0.72	1		
RH	-0.22	0.21	-0.24	1	
THI	-0.17	0.14	-0.23	0.44	1

Table 3a. Correlation among the semen quality parameters of Barbari goat buck in monsoon seasons

	Vol	Conc	SPE	IM	LIV%	AI	Ab	HOST	SPD
Vol	1								
Conc	-0.16	1							
SPE	0.54	0.69	1						
IM	-0.49	0.21	-0.25	1					
LIV%	0.33	-0.56	-0.34	-0.41	1				
AI	-0.61	0.23	-0.16	-0.21	0.08	1			
Ab	0.44	-0.86	-0.41	-0.16	0.69	-0.35	1		
HOST	-0.28	-0.10	-0.39	0.39	0.50	0.34	0.34	1	
SPD	-0.13	-0.04	0.02	-0.39	0.24	0.73	0.14	0.38	1

Table 3b. Correlation among the semen quality parameters of Barbari goat buck in dry summer seasons

	Vol	Conc	SPE	IM	LIV%	AI	Ab	HOST	SPD
Vol	1								
Conc	0.70	1							
SPE	-0.001	0.04	1						
IM	-0.20	0.02	-0.71	1					
LIV%	0.15	0.21	0.33	-0.05	1				
AI	0.92	0.88	-0.03	0.01	0.25	1			
Ab	0.80	0.67	0.39	-0.16	0.39	0.84	1		
HOST	0.10	0.61	-0.18	0.41	0.60	0.41	0.16	1	
SPD	0.21	-0.22	0.60	-0.36	0.051	0.84	0.52	0.62	1

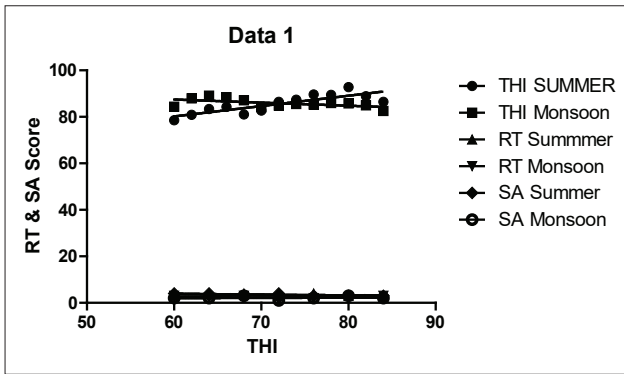


Fig. 2 Correlation graph among the THI and reproductive performance (RT & SA) of Barbari goat buck in dry summer and monsoon seasons

a negative correlation ($P < 0.05$). The correlation analysis exhibited an inverse relationship during the monsoon season (Fig. 2 and Table. 4a).

All these parameters are significantly correlated (≤ 0.05) with each other. RT: Reaction time, SA: Sexual aggressiveness, Tdb: Temperature of dry bulb, RH%: Relative humidity in %, THI: Temperature humidity index. Dry summer season: March to June.

The present study, through the analysis of sexual behavior and spermogram, demonstrated that while only a few environmental factors may affect semen parameters, bucks raised in tropical climates exhibited a degree of seasonality in their reproductive patterns. Our observations indicated that the ejaculate volume was affected by seasonal variations (Barbas *et al.* 2024; Pascal *et al.* 2024). Nonetheless, individual motility and sperm concentration demonstrated a significance variation between summer and monsoon months (Golher *et al.* 2018; Atara *et al.* 2019; Ngcobo, 2023), whereas sperm liability was also elevated during the monsoon season (Isnaini *et al.* 2020). Earlier, investigations has also indicated that the season had a negligible impact on semen ejaculates in bucks, while sperms (millions/mL) were markedly elevated throughout the monsoon season (D'Alessandro *et al.* 2001; Maina, *et al.* 2006; Sultana, 2013). In a similar vein, Elsheikh and Elhammali (2015) noted that individual progressive motility in bucks was elevated during the monsoon season while it decreased in the summer. Numerous studies indicated that the ideal semen reference criteria were achieved exclusively in the fall, whereas the unwanted characteristics were observed in wintertime (Wang *et al.* 2015). The integrity of the acrosomal and plasma membranes revealed the biochemical framework of the sperm plasma membrane, playing a crucial role in various processes such as the acrosomal reaction, capacitation, and the binding of spermatozoa to the oocyte. During summer, thermal stress elevates testicular temperature, subsequently leading to a decline in the integrity of both the acrosomal and plasma membranes, akin to the effects observed with scrotal insulation in goats (Ramu and Jeyendran, 2012). Sperm abnormalities typically normalize within two

months following scrotal thermal insulation (Kastelic *et al.* 1996); however, the recovery rate of these abnormalities is influenced by the duration of exposure during the summer season.

The findings of the current investigation collectively indicated significant differences in sexual behavior and sperm quality parameters between the summer dry and monsoon seasons. This suggests that the influence of seasonal variations is particularly pronounced regarding reproductive behavior and spermogram in Barbari bucks raised in equatorial Indian hot and humid conditions. In summary, the barbari bucks exhibit a more adaptive physiological mechanism for tolerating thermal stress, resulting in certain parameters being non-significant. The reproductive performance, seminal attributes, and sperm functions may be affected by a multitude of factors, such as age, breed variations, nutritional status, overall health, diverse environmental conditions, and the management practices applied to bucks.

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