

RESEARCH ARTICLE

Seasonal dynamics of scrotal infrared profiling and its relationship with seminal attributes of Murrah buffalo bulls

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Abstract: Study on relationship between infrared thermography (IRT) and semen quality, as well as the seasonal variation affecting and the documented reports on the influence of seasons on these relationships are scanty for Murrah bulls (*Bubalus bubalis*). Therefore, goal of experiment was to use IRT as a tool for evaluating seasonal influences on scrotal thermal profiles and its relationship with seminal traits in Murrah bulls. The study was conducted over year, encompassing five distinct seasons: hot dry, hot humid, autumn, winter and spring season, using healthy adult Murrah bulls (N=8). Environmental parameters were recorded to calculate Temperature Humidity Index (THI). Skin surface temperature at various anatomical sites (ear, eye, muzzle, forehead, flank and rump) along with scrotal surface temperature at distal pole, middle pole and proximal pole was recorded with the help of Infrared thermography. Semen ejaculates were collected using an Artificial Vagina. Skin surface temperature at different anatomical sites were significantly ($p<0.05$) lower during winter followed by spring, autumn, hot dry and hot humid seasons. A significantly ($p<0.05$) higher scrotal gradient was observed during winter compared to other season. Seminal parameters including ejaculate volume, sperm concentration, mass motility, progressive motility, viability, hypo-osmotic swelling test (HOST) and acrosomal integrity, were significantly ($p<0.05$) higher during autumn and spring season than other seasons. These findings highlight the importance of considering seasonal variation in scrotal gradient into account when creating breeding

strategies. Optimizing these parameters can improve the semen quality and enhance reproductive efficiency of buffaloes.

Key words: Murrah bulls; Infrared Thermography; Scrotal gradient; Season; Semen.

Introduction

India, with its diverse agro-climatic conditions, hosts one of the largest livestock populations globally, count of 535.78 million (20th Livestock Census) including 109.85 million buffaloes, making a 1.1% increase. While the female buffalo population rise by 8.61%, the male buffalo declined by 42.35%. Buffaloes contribute approximately 58% to the global buffalo population, underscoring their economic and agricultural importance. Buffaloes are highly valued in India for their contributions to milk, meat, and draft power. Among buffalo breeds, Murrah is globally recognized for its superior milk production (Ramajayan et al. 2022), adaptability, and utility as an improver breed. Predominantly raised in Haryana and neighbouring states like Punjab, Uttar Pradesh, and Delhi. Murrah buffaloes are integral to rural livelihoods (ICAR-NBAGR, <https://nbagr.icar.gov.in/wp-content/uploads/2020/02/Murrah-Buffalo.pdf>). However, buffaloes are particularly susceptible to heat stress due to their dark skin, sparse hair coat, and limited sweat gland density compared to zebu cattle, making thermal stress a significant challenge (Debbarma 2018; Bertoni et al. 2020; Umar et al. 2021).

Thermal stress adversely affects buffalo bull's physiological and reproductive performance, particularly during high ambient temperatures (Debbarma, 2018; Bertoni et al. 2020). Heat stress disrupts thermoregulation, leading to reduced feed intake, growth rate, nutrient utilization, and reproductive efficiency (Vaidya et al. 2012; Kumar et al. 2019). In bulls, elevated temperature impairs semen production and quality by affecting parameters such as sperm motility, viability, concentration, and structural integrity. These changes directly influence reproductive efficiency, posing challenges for breeding programs.

IRT has emerged as a non-invasive tool for assessing thermal stress by detecting surface temperature variations (Wijffels et al. 2021). Scrotal temperature is a critical determinant of sperm quality,

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as optimal testicular thermoregulation is essential for spermatogenesis (Kastelic, 2014; Silva et al. 2018). Seasonal variations affect scrotal thermal profiles and, consequently, seminal traits (Ahirwar et al. 2017). Understanding these relationships can provide valuable insights into mitigating heat stress and improving reproductive performance. The aim of the present study was to evaluate seasonal influences on scrotal thermal profiles of Murrah buffalo bulls using infrared thermography and to explore its relationship with seminal characteristics. By analysing these parameters across different seasons, the study seeks to contribute to the development of effective management strategies for enhancing reproductive efficiency of buffaloes under varying climatic conditions.

Materials and Methods

The present investigation was carried out at Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal (Haryana).

Housing and management of bulls

Eight healthy sexually mature Murrah bulls (3-4 years) were selected based on body weight and health status. The study was conducted across five seasons: Hot Dry (April to June), Hot Humid (July to Mid-September), Autumn (Mid-September to November), Winter (December to January) and Spring season (February-March). Bulls were maintained under uniform feeding (ICAR-2013) and management conditions. Vaccination, deworming and other herd-health programme were followed as per the farm schedule to ensure good health.

Recording of climatic variables

Microclimatic data including dry and wet bulb temperature, minimum and maximum temperature, and relative humidity were recorded daily at 9.00 and 15.00 hours using Zeal thermometer [Zeal Masons Pattern Hygrometer P2506 (UK)] throughout the experimental period. The Temperature Humidity Index (THI) was calculated using the National Research Council (NRC, 1971) formula:

$$THI = 0.72 \times (T_{db} + T_{wb}) + 40.6$$

where T_{db} = dry bulb temperature ($^{\circ}\text{C}$),

T_{wb} = wet bulb temperature ($^{\circ}\text{C}$)

Infrared thermography of different anatomical sites and scrotal surface

The temperature of different anatomical sites (eye, ear, muzzle, forehead, flank, rump, and scrotal surface) of bulls during morning and afternoon hours were recorded using the digital IR camera (Therma CamTM SC2000; FLIR Systems, Inc., Wilsonville, OR,

USA) from a distance of about 1 meter and height of about 0.5 to 1.5 meter. Before taking images the body surface and scrotal area was made free from dung and manure. The thermal profile of the scrotum was measured by positioning the camera perpendicular to the scrotum. Temperature was measured at three points of the scrotum i.e. proximal pole temperature (PPT), mid pole temperature (MPT) and distal pole temperature (DPT) (Menegassi et al. 2015). Scrotal gradient (SG) was calculated as difference between PPT and DPT. High quality, focused IRT images were selected for analysis using the Therma CamTM Researcher 2001 software (FLIR Systems AB, Danderyd, Sweden) and an average temperature of each point was recorded. Thermal imaging was conducted at fortnight interval across the seasons.

Semen collection and evaluation

Murrah bulls were given exercise for one hour prior to semen collection in the rotatory bull exerciser to maintain the physical health and sexual vigour. The bulls were thoroughly washed and groomed at least half an hour before semen collection. Semen was collected at weekly interval using bull specific Artificial Vagina (IMV Technologies, France) method, with standard semen collection procedure. Semen collection was performed by well trained and experienced persons. Immediately after collection, the ejaculates were brought in the laboratory and placed in water bath maintained at 32°C for assessing ejaculate volume by measuring in sterilized graduated glass tube, sperm concentration (million/ml) by photometer (IMV Technologies, L'Aigle, France), mass motility (score) by phase contrast microscope (Tomar et al. 1966), progressive motility by phase contrast microscope, sperm acrosome integrity (%) by giemsa staining (Hancock, 1952), hypo-osmotic swelling test (HOST) by using HOST solution (Jeyendran et al. 1984), sperm viability (%) by eosin – nigrosine staining (Campbell et al. 1953) and sperm abnormality (%) by phase contrast microscope (Campbell et al. 1953).

Statistical analysis

To assess the effect of different seasons on various anatomical skin surface temperature and semen quality of Murrah bulls, data was analyzed by one way analysis of variance (ANOVA) using SAS software version 20. Pairwise comparisons of means were carried out using the post-hoc Tukey B test.

Results and Discussion

Meteorological data

The average values of the THI calculated during different seasons from April 2023 to March 2024 are presented in Figure 1. During hot dry season, the THI ranged from 77.59 ± 0.53 in the morning to 86.47 ± 0.48 in the afternoon, with an overall average value of 82.03 ± 0.48 . During the hot humid season, the calculated THI varied from 79.51 ± 0.21 in the morning to 84.01 ± 0.28 in the afternoon, with overall average of 81.76 ± 0.20 . During autumn

season, THI values were 67.59 ± 0.78 in morning and 77.62 ± 0.54 in afternoon with overall average of 72.60 ± 0.63 . During winter, the THI ranged from 52.62 ± 0.36 in the morning to 62.83 ± 0.64 in the afternoon, with an overall mean of 57.72 ± 0.43 . During spring season, the THI increased, with morning values of 63.8 ± 0.84 and afternoon values of 75.36 ± 0.67 , resulting in an overall average of 69.58 ± 0.74 .

The THI is a widely used indicator for assessing heat stress on livestock, as it integrates the combined effects of ambient temperature and relative humidity (Alhussien and Dang, 2017). According to the NRC (1971), animals are considered to be within the thermoneutral zone when the THI is >72 , indicating an absence of heat stress. The classification system proposed by Armstrong (1994) further refines heat stress categories, defining no stress (THI < 72), mild stress (THI 72–78), moderate stress (THI 79–88), severe stress (THI 89–98), and lethal stress (THI > 98). Many studies have reported significantly higher THI values during the hot dry (Kumar and Singh, 2020; Somagond et al. 2021) and hot humid seasons (Deshpande et al. 2020), whereas lower THI values were observed during winter (Deka et al. 2019; Meena et al. 2023) compared to autumn and spring (Chaudhary et al. 2015; Ahirwa et al. 2017; Ahirwa et al. 2018). Elevated THI levels have been shown to negatively impact Murrah bull semen quality, as heat stress disrupts testicular thermoregulation, spermatogenesis, and sperm membrane integrity. Therefore, accurate THI classification is essential for assessing thermal stress and developing effective heat stress mitigation strategies in buffalo breeding programs.

IRT of various anatomical sites and scrotal surface

The skin temperature of various anatomical sites of Murrah bulls were significantly ($p < 0.05$) influenced by the seasonal changes during morning and afternoon hours (Table 1 and 3; Figure 2a to 2j). The highest ($p < 0.05$) skin temperature across multiple anatomical sites (eye, muzzle, ear, forehead, flank and rump) were recorded during hot humid and hot dry compared to other seasons (Table 1). This trend was observed consistently in both morning and afternoon hours. Similarly, scrotal surface temperature at PPT, MPT and DPT regions were significantly ($p < 0.05$) elevated during hot humid and hot dry season compared to other seasons (Table 2; Figure 2b, 2d, 2f, 3h and 2j). The SG, which represents the thermoregulatory efficiency of the scrotum, also exhibited significant ($p < 0.05$) seasons variation. Two-tailed t-test correlation analysis revealed a significant positive correlation ($p < 0.01$) of THI with skin temperature of different anatomical sites and scrotal surface of Murrah bulls (Table 3).

The results demonstrate notable differences in various anatomical site temperature across seasons, with higher values observed during the hot humid and hot dry season, likely due to elevated environmental heat stress. These results are in accordance with Breko et al. (2020), Chikkagoudara et al. (2020) and Vilela et al. (2022) who reported IRT of the eye can serve as a reliable proxy for core body temperature. The eye region exhibited the highest surface temperature (George et al. 2014; Arfuso et al. 2022) and the most suitable site for thermal assessment (Cuthbertson et al. 2019; Souto et al. 2021). Montanholi et al. (2008) demonstrated that thermographic imaging of anatomical sites such as the eye, muzzle (Mota-Rojas et al. 2021), and neck can serve as indicators of alterations in blood flow, which are

Fig. 1 Heat map of THI throughout the experimental period

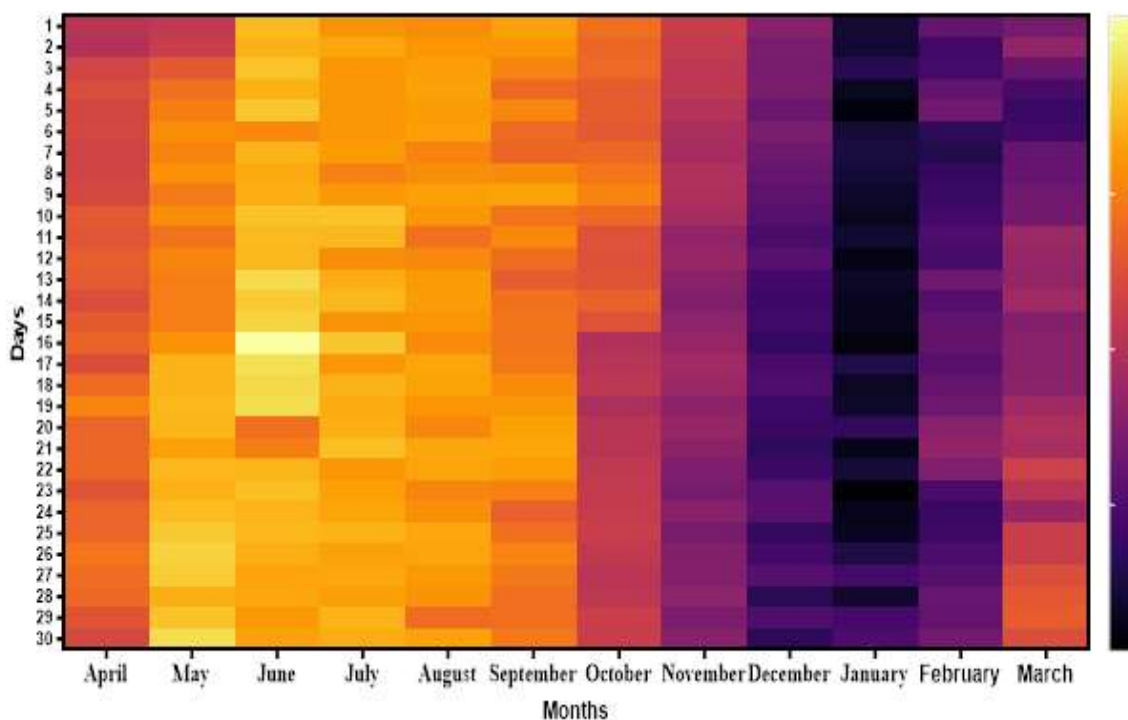


Table 1: Infrared Thermography of different anatomical sites of Murrah bulls during different seasons

Parameters	Eye (°C)		Muzzle (°C)		Ear (°C)		Forehead (°C)		Flank (°C)		Rump (°C)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Hot Dry	35.84 ^z ±0.31	36.83 ^y ±0.18	32.71 ^z ±0.28	34.79 ^z ±0.26	32.80 ^z ±0.37	33.61 ^z ±0.26	34.51 ^z ±0.48	36.36 ^z ±0.30	35.07 ^z ±0.31	37.28 ^z ±0.23	34.42 ^z ±0.41	36.92 ^y ±0.33
Hot Humid	36.33 ^z ±0.13	37.91 ^z ±0.12	33.94 ^z ±0.19	34.80 ^z ±0.20	32.84 ^z ±0.22	34.10 ^z ±0.18	34.75 ^z ±0.29	38.44 ^z ±0.16	34.12 ^z ±0.16	37.11 ^z ±0.18	34.77 ^z ±0.19	37.26 ^z ±0.21
Autumn	33.14 ^y ±0.27	35.37 ^x ±0.23	29.16 ^y ±0.85	33.74 ^z ±0.50	27.41 ^y ±0.64	29.78 ^y ±0.61	29.37 ^y ±0.62	35.44 ^y ±0.46	31.25 ^y ±0.49	36.41 ^y ±0.30	31.61 ^y ±0.58	37.14 ^z ±0.29
Winter	27.74 ^w ±0.37	32.15 ^w ±0.36	21.61 ^x ±0.62	24.82 ^x ±0.68	16.16 ^x ±0.72	17.72 ^x ±0.72	19.39 ^x ±0.48	27.50 ^z ±1.00	23.62 ^w ±0.58	30.39 ^x ±0.75	23.34 ^w ±0.41	30.22 ^x ±0.64
Spring	30.82 ^x ±0.54	35.22 ^x ±0.38	28.25 ^y ±0.39	30.89 ^y ±0.47	28.15 ^y ±0.51	29.96 ^y ±0.54	30.92 ^y ±0.66	35.22 ^y ±0.62	29.67 ^x ±0.42	35.11 ^z ±0.36	30.05 ^x ±0.67	35.66 ^y ±0.36

Different superscript (w, x, y, z) in column depicts significant (p<0.05) difference between seasons.

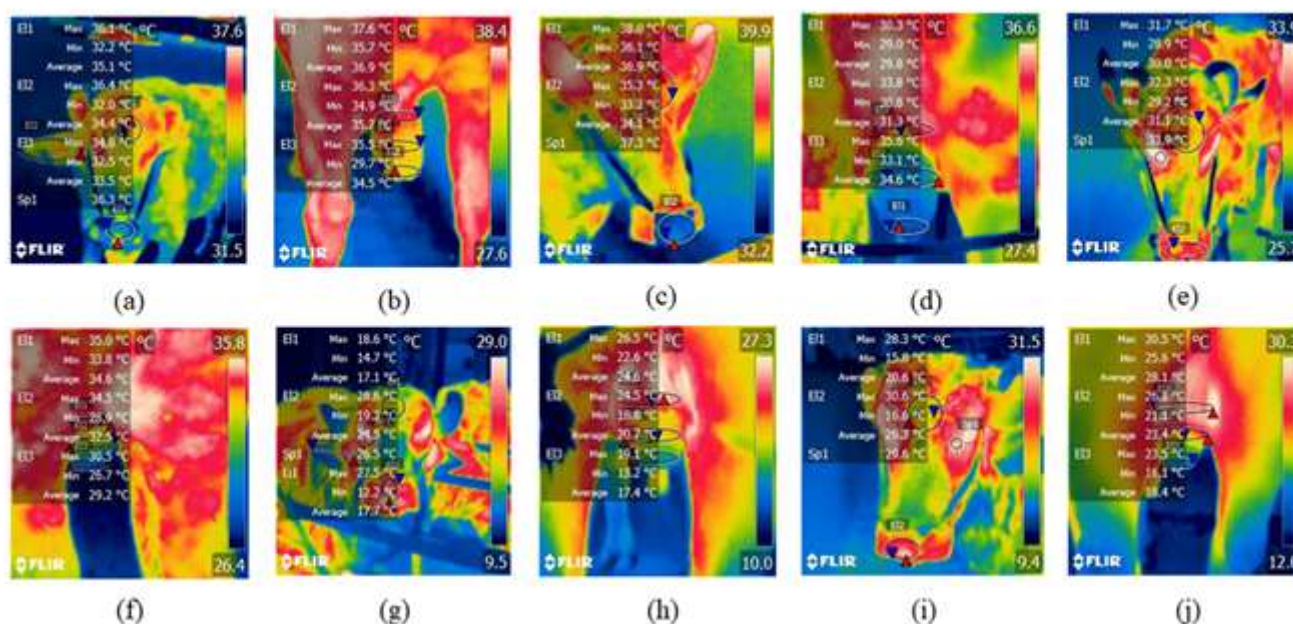
Table 2: Infrared Thermography of different points of scrotum of Murrah bulls during different seasons

Seasons	Proximal pole (°C)		Middle (°C)		Distal pole (°C)		Testicular Gradient (°C)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Hot Dry	34.29 ^z ±0.19	35.16 ^y ±0.14	32.54 ^z ±0.19	33.53 ^z ±0.11	30.36 ^z ±0.22	31.92 ^z ±0.16	3.94 ^{wx} ±0.19	3.24 ^x ±0.16
Hot Humid	34.95 ^z ±0.17	36.23 ^z ±0.19	33.43 ^z ±0.17	34.57 ^z ±0.16	31.46 ^z ±0.14	32.89 ^z ±0.19	3.49 ^w ±0.14	3.34 ^x ±0.18
Autumn	32.09 ^y ±0.21	33.90 ^x ±0.20	29.61 ^y ±0.30	31.91 ^y ±0.21	26.78 ^x ±0.38	29.75 ^y ±0.23	5.31 ^y ±0.29	4.16 ^x ±0.20
Winter	28.92 ^w ±0.27	30.20 ^w ±0.40	23.98 ^w ±0.30	27.58 ^x ±0.64	19.78 ^w ±0.30	24.24 ^x ±0.79	9.14 ^z ±0.23	5.97 ^y ±0.53
Spring	31.11 ^x ±0.41	33.45 ^x ±0.25	27.29 ^x ±0.47	31.19 ^y ±0.31	26.47 ^x ±0.40	29.21 ^y ±0.31	4.65 ^{xy} ±0.33	4.24 ^x ±0.28

Table 3: Correlation of THI with skin temperature of different anatomical sites of Murrah bulls during different seasons

	THI	Forehead (°C)	Eye (°C)	Muzzle (°C)	Ear (°C)	Flank (°C)	Rump (°C)	Proximal pole (°C)	Middle pole (°C)	Distal pole (°C)
THI	1									
Forehead (°C)	0.820 ^{**}	1								
Eye (°C)	0.878 ^{**}	0.846 ^{**}	1							
Muzzle (°C)	0.847 ^{**}	0.828 ^{**}	0.835 ^{**}	1						
Ear (°C)	0.854 ^{**}	0.876 ^{**}	0.833 ^{**}	0.808 ^{**}	1					
Flank (°C)	0.829 ^{**}	0.887 ^{**}	0.827 ^{**}	0.807 ^{**}	0.873 ^{**}	1				
Rump (°C)	0.836 ^{**}	0.868 ^{**}	0.832 ^{**}	0.855 ^{**}	0.839 ^{**}	0.895 ^{**}	1			
Proximal pole (°C)	0.869 ^{**}	0.784 ^{**}	0.808 ^{**}	0.851 ^{**}	0.771 ^{**}	0.766 ^{**}	0.813 ^{**}	1		
Middle pole (°C)	0.913 ^{**}	0.833 ^{**}	0.841 ^{**}	0.898 ^{**}	0.816 ^{**}	0.817 ^{**}	0.860 ^{**}	0.938 ^{**}	1	
Distal pole (°C)	0.898 ^{**}	0.904 ^{**}	0.877 ^{**}	0.889 ^{**}	0.870 ^{**}	0.881 ^{**}	0.897 ^{**}	0.958 ^{**}	0.902 ^{**}	1

^{**}(p<0.01)



(a) Eye, forehead, muzzle hot dry season (b) scrotum during hot dry season (c) eye, forehead, muzzle during hot humid (d) scrotum during hot humid (e) eye, forehead, muzzle during autumn (f) scrotum during autumn (g) eye, forehead, muzzle during winter (h) scrotum during winter (i) eye, forehead, muzzle during spring (j) scrotum during spring

Fig. 2 Infrared Thermograms of different anatomical sites of Murrah buffalo bulls during different seasons

associated with elevated body temperature under stressful conditions. During the hot humid season, high relative humidity impairs evaporative cooling efficiency by limiting sweat and respiratory water loss. Since buffaloes have a limited number of sweat glands and primarily rely on cutaneous and respiratory heat loss, high humidity impairs heat dissipation, causing skin temperature to remain elevated. Conversely, during the hot dry season, despite high ambient temperature the lower humidity facilitates better evaporative cooling. However, heat stress still leads to increased skin temperature compared to cooler seasons. In response peripheral blood vessels, particularly in exposed body regions (muzzle, ear, and forehead) undergo vasodilation, increasing blood flow to facilitate heat dissipation via radiation, convection, and evaporation (Mader et al. 2010). This thermoregulatory response leads to significantly higher skin temperature during the hot humid and hot dry seasons. Elevated environmental temperature and the increased heat load contribute to higher heat gain from the surroundings, with the skin acting as a primary heat exchange surface. The combination of high ambient temperature and solar radiation intensifies cutaneous heat dissipation (Mader et al. 2010). In contrast, during the winter season, lower environmental temperature reduced external heat load and vasoconstriction minimize heat loss by restricting blood flow to the periphery, leading to lower skin surface temperature. The non-invasive nature of IRT allows for repeated, stress-free assessments, making it suitable for routine monitoring in breeding programs. This approach may aid in identifying bulls at risk of

reduced fertility during peak stress periods, enabling targeted management interventions. The THI exceeding critical thresholds during hot dry and hot humid season correlates with increased thermal load on the scrotum, compromising thermoregulation (Yadav et al. 2019). Our finding aligns with those of Menegassi et al. (2015) and Ahirwar et al. (2017) who also reported decreased SG temperature during summer (hot dry and hot humid season) compared to winter season. Elevated scrotal temperature likely impairs spermatogenesis and epididymal sperm maturation, leading to reduced semen quality. In contrast, optimal thermal profiles recorded during autumn and spring correspond with improved seminal parameters, highlighting the influence of ambient temperature on testicular function. Our findings align with Barros et al. (2016) who reported positive correlation between THI and temperature in the orbital area (0.72), right flank (0.77), left flank (0.75) and scrotum (0.41) in Buffalo bulls. We found significant positive correlation ($P < 0.01$) of THI with skin temperature of different anatomical sites and scrotal surface of Murrah bulls which are in accordance with Ahirwar et al. (2018) and Somagond et al. (2021) who found significant positive correlation of THI with scrotal surface temperature in Murrah bulls.

Semen quality

Seasonal variations on fresh semen parameters including semen volume (ml), sperm concentration (millions/ml), mass motility (score), progressive motility (%), viability (%), HOST (%) and

Table 4: Seminal characteristics of fresh bull semen of Murrah bulls during different seasons

Seasons	Volume (ml)	Concentration (millions/ml)	Mass motility (score)	Progressive motility (%)	Viability (%)	HOST (%)	Acrosomal Integrity (%)
Hot Dry	3.50±0.16	1399.20 ^{xy} ±28.95	2.45 ^y ±0.06	73.13 ^x ±0.80	79.27 ^x ±0.80	69.43 ^x ±1.11	84.07 ^x ±0.47
Hot Humid	3.63±0.18	1301.83 ^x ±40.13	2.44 ^y ±0.06	72.88 ^x ±0.78	79.90 ^x ±0.67	68.86 ^x ±1.05	82.75 ^x ±0.58
Autumn	3.79±0.07	1450.29 ^y ±21.34	2.77 ^z ±0.05	76.44 ^y ±0.53	84.04 ^z ±0.40	73.89 ^y ±0.73	88.14 ^z ±0.53
Winter	3.37±0.09	1363.67 ^{xy} ±23.62	1.78 ^x ±0.05	74.88 ^{xy} ±0.61	80.85 ^{xy} ±0.70	70.65 ^{xy} ±0.55	85.77 ^y ±0.36
Spring	3.71±0.08	1454.74 ^y ±20.66	2.69 ^z ±0.06	76.80 ^y ±0.64	82.93 ^{yz} ±0.48	73.75 ^y ±1.05	87.87 ^z ±0.45

The Mean±SE of ten observations on eight animals.

Different superscript (x, y, z) in column depicts significant ($p<0.05$) difference between seasons.

acrosomal integrity (%) are presented in Table 4. Among these, mass motility (score), progressive motility (%), viability (%), HOST (%) and acrosomal integrity (%) were significantly ($p<0.05$) lower during hot humid and hot dry seasons compared to autumn season in Murrah bulls.

The present study revealed significant seasonal variations in fresh semen parameters like mass motility (score), progressive motility (%), viability (%), HOST (%) and acrosomal integrity (%) of Murrah bulls. Bhakat et al. (2015) also found significant ($p<0.05$) effect of seasonal variation on fresh semen parameters but no significant difference was found in ejaculate volume in Murrah bulls which was also found by Koonjaenak et al. (2007) and Mishra (2013) in swamp buffalo bulls. Bhave et al. (2020) observed a significantly ($p<0.05$) lower sperm concentration during summer season in Indigenous bulls. Sharma et al. (2018) found no significant effect on ejaculate volume but significant ($P<0.05$) effect was seen in mass motility and progressive motility. They concluded mass motility and progressive motility of the semen was significantly ($p<0.05$) highest at the THI value of 72-78 and lowest at the THI value of 78-84 similar pattern was also observed in these parameters during the present study. These findings highlight the negative impact of elevated ambient temperature on semen quality, which can be attributed to thermoregulatory challenges affecting testicular functions. Testicular thermoregulation is crucial for optimal spermatogenesis in bulls. The testicular vascular cone (TVC), located in the upper region of the testicles, facilitates counter current heat exchange, ensuring that arterial blood is cooled before reaching the testes. This is essential for maintaining testicular temperature 2 to 6°C below core body temperature, (Coulter and Kastelic, 1994; Kushwaha et al. 2018). An increase beyond the physiological range (33–34.5°C) negatively affects sperm production and quality, leading to impaired motility, viability, and morphological abnormalities (Barth and Bowman, 1994). The decline in sperm motility and viability observed in the present study during hot seasons can be linked to the disruption of testicular thermoregulation due to heat stress. Elevated ambient temperature impairs the efficiency of the TVC that negatively

impact spermatogenesis. High testicular temperature can alter mitochondrial function by interfering with the oxidative metabolism of glucose in spermatogenic cells, leading to increased production of reactive oxygen species (ROS) (Kastelic et al. 2018; Bertoni et al. 2020). Excessive ROS generation induces oxidative stress, which damages sperm membranes, proteins, and DNA, thereby reducing sperm motility, viability, and acrosomal integrity. Additionally, exposure to high environmental temperature during the hot humid and hot dry seasons may result in increased scrotal sweating and panting as thermoregulatory responses. However, under prolonged heat stress, these mechanisms may become insufficient, leading to persistent testicular hyperthermia and subsequent sperm damage. This can explain the significant decline in HOST values observed in the present study, as the sperm membrane integrity is compromised. Humidity further exacerbates the effect by reducing evaporative cooling efficiency, thereby increasing rectal temperature and respiratory distress. Prolonged heat stress has also been associated with hormonal imbalances, which further impair spermatogenesis. Moreover, oxidative stress induced by heat exposure accelerates germ cell apoptosis and compromises sperm DNA integrity, contributing to decreased fertility. In contrast, during the autumn and spring season, the relatively lower ambient temperature supports effective testicular thermoregulation, ensuring optimal conditions for spermatogenesis. This is reflected in the higher values of mass motility, progressive motility, viability, HOST, and acrosomal integrity recorded during this season. The favourable thermal environment likely facilitates better mitochondrial function and reduces oxidative stress. Overall, these findings underscore the detrimental impact of heat stress on semen quality in Murrah bulls. The results emphasize the need for strategic management interventions, such as providing heat stress mitigation strategies (e.g., shade, evaporative cooling, dietary antioxidants), to improve reproductive efficiency in breeding bulls under hot environmental conditions.

Conclusion

This study highlights the significant influence of seasonal variations on skin surface temperature at different anatomical sites and scrotal thermal profiles and semen quality in Murrah buffalo bulls. IRT proved to be an effective tool for assessing these changes, demonstrating superior seminal traits during autumn and spring. The findings emphasize the importance of incorporating seasonal variations in breeding strategies to optimize reproductive efficiency. Understanding the relationship between scrotal thermal gradients and seminal characteristics can aid in developing targeted management practices to mitigate heat stress and enhance buffalo breeding programs.

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Data availability

The data that support this study will be made available on reasonable request.

Conflicts of interest

No potential conflicts of interest were reported by the authors.

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