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IMPACT OF TEMPERATURE HUMIDITY INDEX (THI) ON OCULAR-SCROTAL THERMOREGULATION AND SEMEN QUALITY IN HF CROSSBRED BULLS

S. D. Kharde, S. D. Ingole and S. V. Bharucha
Department of veterinary Physiology,
Mumbai Veterinary College, Mumbai
drsdkharde@gmail.com, simin1972@gmail.com

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ABSTRACT:
This study evaluated seasonal thermal stress impacts on thermoregulation and semen quality in 20 Holstein Friesian crossbred bulls in Mumbai. Environmental conditions shifted significantly from a comfortable winter temperature humidity index (THI) of 70.55 to a stressful summer THI of 80.40. Infrared thermography revealed that elevated summer heat load significantly increased systemic ocular temperatures from 97.66 to 99.33. Concurrently, localized scrotal surface temperatures rose across proximal, mid, and distal poles, while the scrotal thermal gradient narrowed from 5.14°C to 4.76°C, indicating compromised testicular thermoregulatory capacity. This thermal breakdown severely impaired summer spermatogenesis. Sperm concentration dropped from 1053.67 x 10^6 ml to 886.54 x 10^6 ml, initial motility decreased from 77.69% to 73.24%, and membrane integrity (HOST) declined from 66.76% to 56.14%. Morphological abnormalities also rose sharply from 10.96% to 16.28%. Monitoring ocular-scrotal thermal profiles provides vital insights for managing breeding bull fertility in tropical environments.

Keywords: Temperature Humidity Index, Infrared Thermography, Semen Quality, Testicular Thermoregulation, HF Crossbred Bulls

INTRODUCTION

In tropical regions like India, maintaining top-tier reproductive efficiency in breeding bulls is a major challenge. While India remains a leading global milk producer, its average milk production per cow (1310 kg) lags behind the world average of 2200 kg (Hegde, 2019). Improving this yield relies heavily on robust breeding programs, which are currently hindered by seasonal drops in semen quality (Cardozo *et al.*, 2006; Bhakat *et al.*, 2014). High summer ambient temperatures and elevated Temperature Humidity Indexes (THI) exceeding the baseline stress threshold of 72 trigger systemic heat stress in dairy cattle (West et al., 2003; Bohmanova et al., 2007).

When ambient conditions deteriorate, non-invasive tools like infrared thermography (IRT) allow researchers to assess a bull's physiological response to environmental changes (Paim et al., 2013). By recording pictorial images of surface infrared emissions without physical contact, IRT can evaluate both ocular and scrotal surface temperatures. In summer, as environmental heat load rises, a bull's core body temperature increases. This systemic rise is directly mirrored by elevated ocular temperatures, which serve as a reliable indicator of internal physiological heat stress. Concurrently, the animal attempts to stabilize its testicular environment. For normal spermatogenesis and the production of fertile sperms, testicular temperatures must be strictly maintained at 2 to 6°C below core body temperature, staying within a narrow window of 33 to 34.5°C (Coulter and Kastelic, 1994; Barth and Bowman, 1994).

As ambient temperatures soar, the correlation between a rising ocular temperature and an elevated scrotal surface temperature highlights a failure or

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strain in the bull's testicular thermoregulatory mechanisms. Infrared thermography demonstrates that an increase in scrotal surface temperature directly reflects a rise in the temperature of the underlying testes and epididymis. When thermal regulation fails during a summer THI of 83, the normal temperature gradient between the proximal and distal scrotal poles decreases, directly correlating with a severe drop in overall sperm quality (Menegassi *et al.*, 2015).

This thermal breakdown causes several distinct reproductive issues including Cellular Damage (Mishra *et al.*, 2013), Reduced Fertilization (Rahman *et al.*, 2013) and Poorer Semen Profiles (Bhakat *et al.*, 2014).

Ultimately, tracking the close relationship between ocular heat indicators and scrotal thermal profiles using IRT helps identify bulls suffering from reproductive failure due to acute climate events like heat waves (Hansen, 2009). Understanding this correlation is vital for protecting the genetic potential of crossbred bulls in tropical environments. This study was aimed to correlate scrotal temperature with ocular temperature in hot and humid region during summer season.

MATERIAL AND METHODS

Animal Selection and Management

A total of 20 healthy Holstein Friesian (HF) crossbred breeding bulls aged 3–8 years were selected from the Konkan Development Corporation Ltd. (Aarey Colony, Goregaon East, Mumbai). The animals were housed under a conventional, East-West oriented shed featuring concrete flooring, a central passage, individual mangers, and dedicated watering spaces.

Bulls were fed a daily concentrate ration containing 21% Crude Protein (C.P.) and 70% Total Digestible Nutrients (TDN) at 2.5 kg per bull. This was supplemented with locally available seasonal green fodder (paragrass, napier, or ragi straw) and ad libitum clean drinking water. To maintain libidos and preserve semen quality, bulls underwent rotatory exercise twice weekly, one day prior to scheduled collections. Herd health, including routine vaccinations and deworming, followed strict farm schedules.

Environmental Monitoring

The microclimate inside the shed was tracked continuously using a National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited dry and wet bulb thermometer during winter (Nov.-Feb.) and summer (March–June).

The Temperature Humidity Index (THI) was calculated using the National Research Council formula:

THI = (Db + Wb) \times 0.72 + 40.6

Where Db represents the dry bulb temperature and Wb represents the wet bulb temperature. Seasonal mean THIs were recorded at 70.55 (winter), and 80.40 (summer). Thermal stress environments were classified by standard deviations from the overall mean: Low (THI < 70.06), Medium (THI 70.06 to 80.88), and High (THI > 80.88).

Infrared Thermography (IRT)

Thermographic profiling of the orbital and scrotal regions was performed biweekly in the morning (06:00 to 11:00 hrs) using a calibrated FLIR E6 infrared camera. Readings spanned two consecutive spermatogenic cycles (36 days total) per season.

- **Scrotal Imaging:** Scrotal skin was wiped clean of mud and debris. Perpendicular images were taken 1 meter away at scrotum level. FLIR Quick Report software was used to analyze the proximal (PPT), mid (MPT), and distal (DPT) pole temperatures. The thermal gradient (TG) was calculated as the difference between PPT and DPT extremities.
- **Ocular Imaging:** Lateral images were captured 1 meter from the head. A circle was drawn around the orbital region to isolate the capillary-rich ocular globe and monitor systemic thermal status.

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Out-of-focus images caused by animal movement were excluded from final analysis.

Semen Collection and Evaluation

Ejaculates were collected via an artificial vagina (AV) maintained at 42–45°C with appropriate internal pressure and lubrication. Samples were immediately placed in a 37°C water bath for analysis using standard Bhosrekar methods (2005). The Volume, pH, Color, Density, Mass Activity & Initial Motility, Concentration & Viability and Hypo-Osmotic Swelling Test (HOST) were tested as per standard procedures.

Statistical Analysis

Data regarding scrotal and ocular surface temperatures, seasonal THI, and semen characteristics were analyzed via one-way ANOVA using SPSS software. Group means were compared using Duncan's multiple range test at 95% and 99% significance levels ($P < 0.05$ and $P < 0.01$). Trait interactions were calculated using Pearson's correlation coefficients.

RESULTS

The environmental conditions changed substantially between seasons. Results are indicated in table No. 1. The THI increased significantly from a comfortable 70.55 ± 0.37 in winter to a stressful 80.40 ± 0.25 during the summer. This elevated environmental heat load corresponded with a notable rise in systemic and localized physiological temperatures:

Ocular Temperature (OcT): Elevated significantly from 97.66 ± 0.19 in the winter to 99.33 ± 0.12 in the summer, serving as an indicator of systemic heat stress.

Scrotal Surface Temperatures: All measured points on the scrotum increased markedly during the summer. The proximal pole temperature (PPT) rose from 95.82 ± 0.17 to 97.46 ± 0.11, the mid pole temperature (MPT) rose from 93.82 ± 0.16 to 95.31 ± 0.11, and the distal pole temperature (DPT) increased from 91.24 ± 0.21 to 92.71 ± 0.13.

Thermal Gradient (TG): The temperature differential between the proximal and distal extremities narrowed from 5.14 ± 0.55 in winter to 4.76 ± 0.11 in summer, showing a compromised testicular thermoregulatory capacity during hotter months.

Semen Quality

The physical and microscopic attributes of the collected ejaculates were visibly impacted by these elevated summer temperatures.

Ejaculate Volume, Density, and pH

The average ejaculate volume remained statistically stable between the seasons, measuring 4.08 ± 0.06 ml in winter and 4.12 ± 0.03 ml in summer. However, semen density dropped significantly from a higher score of 3.13 ± 0.03 in the winter to 2.84 ± 0.03 during the summer. Semen pH exhibited a minor decrease from 6.60 ± 0.02 in winter to 6.52 ± 0.02 in summer.

Sperm Concentration and Mass Activity

Sperm concentration experienced a severe decline under summer heat stress, dropping significantly from 1053.67 ± 7.74 10^6 ml in the winter down to 886.54 ± 7.21 \times 10^6 ml in the summer. Conversely, mass activity values did not show a statistically significant difference between winter (3.77 ± 0.04) and summer (4.50 ± 0.52 NS).

Motility, Viability, and Membrane Integrity

Summer heat stress severely impaired the functional characteristics of the spermatozoa.

Initial Motility: Fell significantly from 77.69 ± 0.19% in winter to 73.24 ± 0.53% in summer.

Live Sperm Percentage: Declined significantly from 79.69 ± 0.17% during the winter to 75.55 ± 0.16% during the summer.

Abnormal Sperm Percentage: Increased drastically from 10.96 ± 0.15% in winter to 16.28 ± 0.27% in summer, indicating morphological damage during summer spermatogenesis.

Hypo-Osmotic Swelling Test (HOST): Sperm biochemical membrane integrity was highly compromised in the summer, dropping significantly

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to 56.14 ± 0.23% compared to the optimal 66.76 ± 0.27% recorded in winter.

DISCUSSION

The overall mean of semen ejaculate volume (ml) during winter, and summer seasons were 4.08 ± 0.06, and 4.12 ± 0.03, respectively. However, it was observed that there was no significant difference between different seasons on semen ejaculate volume. Similar non significant results were reported by Bhakat *et al.* (2014), and Mishra *et al.* (2012) in KF, Jersey, and Nellor bulls respectively. Comparatively the ejaculate volume in the present study was lower than the findings of Bhosrekar *et al.* (1980) in Jersey and HF bulls and higher to the reports of Dhami *et al.* (1998) and Tiwari *et al.* (2010) in Murrah buffalos.

The average density of fresh semen ejaculate collected from HF cross bulls was 3.13 ± 0.03, and 2.84 ± 0.03 during winter, and summer seasons respectively. It was observed that the semen density differed significantly (P<0.01) within the different seasons. The highest significant semen density was observed in winter and lowest in summers. Similarly, Sarder (2007) also found significant difference in semen density of HF bulls during the different seasons, but reported high density during summer than in winter which is in contrast to the present study. No significant variation was reported by Koonjaenak *et al.* (2007) in swamp buffaloes. These variations in density may be attributed to ambient temperature humidity, location, environmental and managemental factors.

The average semen pH of fresh semen ejaculate collected from HF cross bulls were 6.60 ± 0.02, and 6.52 ± 0.02 during winter, and summer season, respectively. The mean pH of semen was non-significantly low (6.52 ± 0.02) during summer season. These results are in accordance with Bhakat *et al.* (2014), Hossain *et al.* (2012) and Koonjaenak *et al.* (2007) in KF, HF and swamp buffalo bulls.

The colour of the fresh ejaculated semen of HF cross bulls were white in colour during winter and creamy white in summer season, respectively. These results are in accordance with Andre *et al.* (2017), Kumar *et al.* (2015) in HF, and Jersey, respectively.

The overall mean of sperm concentration (×106 / ml) during winter, and summer seasons were 1053.67 ± 7.74, and 886.54 ± 7.21, respectively. In the present study, sperm concentration was significantly (P<0.01) higher in the winters than summer season. The sperm concentrations were in accordance with Santoso *et al.* (2021), Prastowo *et al.* (2019, 2018), Chikhaliya *et al.* (2018), Argiris *et al.* (2018) in Pasundan bull, Bali, HF, Jersey bulls.

The dead and abnormal spermatozoa were more during summer as compared to winter. These are absorbed through phagocytosis and increased resorption of abnormal spermatozoa leads to reduction in epididymal sperm reserves and ultimately decreases in concentration (Bhakat *et al.* 2014).

The non-significant variation of mass activity in the present study is in consonance with Santoso *et al.* (2021), Sharma (2018), Chikhaliya *et al.* (2018), Kumar *et al.* (2015), Kanchan and Matharoo (2015), Bhakat *et al.* (2014 and 2011), Fiaz *et al.* (2010), Koivisto *et al.* (2009) and Koonjaenak *et al.* (2007) in Pasundan bull, HF, Jersey, Gir, KF, Sahiwal bull respectively. The environmental temperature or THI had no significant influence on mass activity. This has an important attribute for acceptance or rejection of ejaculate for further processing. The non-significant variation in mass activity may be due to variation in degree of sexual excitement, age of bull

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and breed characteristics (Kumar *et al.* 2015). Such mass activity across season indicates that seasonal changes had no effect on testicular production throughout the year.

Significant decrease in initial motility during summers is due to the ability of homeotherms in maintaining constant body temperature which is compromised when environmental conditions limit the loss of metabolic heat or contribute to the heat load of animal. The heat stress affects animal regulation of body temperature and ultimately semen production and motility (Sharma, 2018). The decrease in motility may arise due to acids from anerobic sperm metabolism which inhibits sperm movement (Kumar *et al.* 2015). The high temperature and hot winds discomforts the animal and thus reduction in feed and fodder of bulls leading to thermal insult of testicular epithelium (Mishra *et al.* 2012).

Significantly higher values of live sperm % were noted in winter season followed by in the summer season. The significantly high percent sperm viability during winter and low in summer season is in accordance with Sharma *et al.* (2017), Andre *et al.* (2017) and Mishra *et al.* (2013) in crossbred, HF, Jersey, Red Sindhi bulls. The degenerative changes in seminiferous tubules and testicular degeneration due to thermal stress causes lower viability and output during summers (Andre *et al.* 2017). The disturbed spermatogenesis due to high environmental temperature the percent sperm viability is low in summers than in cold (Mishra *et al.* 2013).

In the present study highly significant (P<0.01) difference were observed in abnormal sperm % during winter, and summer season. The highest abnormal sperm % was recorded in summer season and lowest in winter season. The similar significant pattern of abnormal sperm % recorded in the present study is in agreement with Nongbua *et al.* (2020), Bhakat *et al.* (2014) and Koivisto *et al.* (2009) in Sahiwal, KF and Nelore bulls. The high environmental temperature increases testicular temperature causing increased ROS and hypoxia. There is increased alkalosis due to increased respiratory rate inhibiting oxygen to the tissues. The increase in ROS concentration causes lipid peroxidation and damage DNA. Thus due to heat stress during spermatogenesis the quality of sperm is decreased and there is increase in abnormal sperms. Besides, sperms during meiosis are affected by scrotal insulation and changes in level of protamination in sperm DNA is highest with high temperature (Morrell, 2020).

HOST % was significantly (P<0.01) lower in the summer season (56.14 ± 0.23) as compared to winter (66.76 ± 0.27). This finding of present study is in agreement with Sharma *et al.* (2017), Bhakat *et al.* (2014) and Mishra *et al.* (2013) in crossbreds, Red Shindhi, Jersey and HF bulls. The sperm plasma membrane is always prone to free radical attack. Due to oxidative stress there is negative effect on spermatogenesis which damages sperm membrane and DNA and thus decreases HOST % in summers or during high temperature. ROS production is enhanced due to heat stress thus disrupting sperm membrane (Sharma *et al.* 2017).

The PPT was significantly (P<0.01) lower in winter than in summer season. This is in accordance with Ahirwar *et al.* (2017), Ruediger *et al.* (2016), Menegassi *et al.* (2015) in Murrah, Nellore and Bradford bulls. Similar result during rainy season was also reported by Silva *et al.* (2018).

It was observed that the MPT was highly significant (P<0.01) during summer than in winters. These results are similar to the findings of Ahirwar *et al.* (2017), Ruediger *et al.* (2016) in Murrah and Nellore bulls.

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The average scrotal surface DPT of HF cross bulls during winter was 91.24 ± 0.21, and in summer 93.33 ± 0.11. Similar to PPT and MPT, DPT was also significantly (P<0.01) lower in winter than in summer season. This result corroborates with Ahirwar *et al.* (2018 and 2017), Ruediger *et al.* (2016) and Menegassi *et al.* (2015) in Murrah, Nellore and Bradford bulls.

The findings of the present study with respect to TG is in agreement with Ahirwar *et al.* (2018, 2017), Menegassi *et al.* (2018, 2016 and 2015) in Murrah, Bradford and Nellore bull. The modification in scrotal TG can be related to testicular damage by heat stress factors or acute inflammatory process (Menegassi *et al.* 2018). A major variation in TG can be an indicative of semen quality and testicular functioning / damage. High TG indicates low scrotal temperature wherein the bulls are comfortable which was observed during winters followed by summers. The present study indicates that scrotal TG decreases in summer as compared to winter season.

The overall mean of ocular temperature (OcT) of HF cross bull during winter, and summer seasons were 97.66 ± 0.19, and 99.33 ± 0.12, respectively. The highest ocular temperature was recorded during summer and lowest during winters. The results of present study are in concurrence with Ahirwar *et al.* (2018, 2017) Menegassi *et al.* (2018, 2016 and 2015) in Murrah, Brangus and Bradford bulls. In contrast Silva *et al.* (2018) did not find any change in ocular temperature with change of season.

Thus it is concluded that the scrotal temperature, climatic factors and THI influence the quality of semen. The bulls evaluated throughout the seasons demonstrated variation in seminal characteristics with change in THI. The scrotal thermography can be adopted as an indirect tool to know the effect of environment by assessing testicular gradients on sperm physical and quantitative characteristics. Further, scrotal thermography may contribute to select more heat resistant bulls and is sufficiently robust for valid assessment in semen station or even in field conditions.

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