RESEARCH ARTICLE

Effect of Heat Stress on Sperm Production, Oxidative Markers and their Association in Native Breeding Bulls

Arjun J Dhami[1], Vishal S Suthar[2*], Dinesh V Chaudhari[1], Kamlesh K Hadiya[1]

Abstract

The objective of the study was to evaluate the effect of climatic conditions on fortnightly semen quality and its oxidative markers, and to evaluate the correlation between them in zebu cattle and buffalo breeding bulls under weekly twice semen collection schedule. The macro- (observatory; daily; n=330) and micro- (bull shed; hourly; n=8540) climatic parameters, *viz.*, ambient temperature (AT °C) and relative humidity (RH %) were measured during Jan to Dec 2018 and temperature humidity index (THI) was generated. There were high associations between micro and macro AT (r=0.982), RH (r=0.897) and THI (r=0.985). Overall, micro-AT was 1.20°C higher, micro-RH was 5.42% lower and the micro-THI was 0.50 higher than the corresponding macro-climatic parameters. Therefore effect of micro-climatic AT, RH and THI was evaluated on biweekly collected 26 ejaculates of three bulls each of Surti, Murrah buffalo and Gir cattle breeds. The macro and microscopic parameters of semen and oxidative markers in seminal plasma, *viz.*, catalase, lipid peroxidation (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined. During the day 72.6% of hours, bulls experienced stress (THI > 72). No impact of season was observed on seminal attributes and oxidative markers of cattle and buffalo bulls, however correlation coefficients were achieved. The results of our study warrants further investigation on more number of bulls including their semen freezability and fertility.

Keywords: Native Bull, Macro-micro-climate, Oxidative markers, Semen production, Tropics.

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Introduction

In livestock farming it is said that "bull is half the herd" meaning it comprises half of the farm genetics. Therefore it is important to choose correct bull to improve farm genetics. The bulls only express best possible phenotypic capabilities when there is good environment. Many plausible factors can affect the semen production and bull fertility. One of growing concern is heat stress due to climate change. India is blessed with the best breeds of indigenous cattle and buffalo with sizable number of crossbreds. However, per head milk production is concern of the day. Although, Indian cattle and buffalo breeds are adapted to heat stress, it causes summer infertility in females and has detrimental effect on testicular function, spermatogenesis, leading to poor sperm morphology, low sperm quality, abnormal DNA/chromatin and endocrine and biochemical alterations in male (Hansen, 2009; Polsky and von Keyserlingk, 2017). Semen quality is influenced by environmental stress factor and seasonal influence. However, limited studies have evaluated effect of heat stress on oxidative markers, *viz.*, catalase, lipid peroxidation (malondialdehide-MDA production), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Nichi *et al.*, 2006; Soren *et al.*, 2016).

It is well established and number of studies have demonstrated the effects of heat stress on female (Ravagnolo *et al.*, 2000; Hansen, 2004) and male (Ram *et al.*, 2017, Verde *et al.*, 2020) fertility using subjective measurements like seasonal periods, defined photoperiods as well as other environment measurements (Bhakat *et al.*, 2011; Polsky and von Keyserlingk, 2017). Many scientists used the Temperature Humidity Index (THI) a measure of heat stress on dairy cattle production and reproduction (Suthar *et al.*, 2012; Schüller *et al.*, 2016; Polsky and von Keyserlingk, 2017). Dikmen and Hansen (2009) suggested that subjective parameters like season cannot predict effect of heat stress on production or reproduction parameters in animal sciences, however THI can. There is dearth of scientific information using quantitative measure, *i.e.*, THI, which may explain the magnitude of effect of heat stress on semen parameters, oxidative indices and bull fertility. Therefore this study was

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planned to evaluate the effect of macro- and micro-climatic conditions on semen quality, oxidative stress markers and to establish the correlations between them in cattle and buffalo bulls.

Materials and Methods

Bull/Semen Lab Parameters:

Three breeding bulls each of Gir cattle and Surti, Murrah buffalo breeds, aged 5.5-11 years, maintained at semen station of Department of Gynaecology and Obstetrics of the Veterinary College, AAU, Anand (Gujarat), India were used. The bulls were housed and managed identically in a semi-loose housing system in separate pen. Bulls were fed on grass and commercial concentrate and minerals supplement. All the bulls were under regular weekly twice semen collection schedule using AV. For this study, the semen production data of these bulls were recorded bi-weekly (26/bull) of the entire year 2018. Ejaculates were evaluated by routine macro-microscopic lab tests. Seminal plasma of bi-weekly/fortnightly ejaculates were stored at -20°C and were used for assessment of oxidative markers, *viz.*, catalase, lipid peroxidation (MDA), SOD and GPx at the end of the year using commercial ELISA kits (Cayman Assay Kits, USA) as per the manufacturer's instructions.

Environmental Parameters

Micro-climate (on farm) data: The maximum, minimum and average ambient temperature-AT (°C) and relative humidity-RH (%) within the bull sheds were recorded every hour using four Tinytag Plus II loggers (Gemini Loggers Ltd., Chichester, UK) that were secured at beams 2 m from the ground and 30 feet apart two in one buffalo shed, one in Gir bull shed and one in collection arena. These loggers measured AT from -25 to +85°C with an accuracy of ±0.3°C and a resolution of 0.01°C and RH from 0 to 100% with an accuracy of ±3% and a resolution of 0.3%.

Macro-climate (from the AAU observatory) data: The corresponding climatic data were collected from nearest climatic observatory of Department of Meteorology, BA College of Agriculture, AAU, Anand, and were correlated with micro-climatic data.

Statistical Analysis

Data on micro-climatic elements were downloaded from Tinytag software in excel files and the macro-climatic elements were captured in excel document. Macro and micro climatic THI scores were calculated according to the equation of Kendall *et al.* (2008) [THI = (1.8 × AT°C + 32) – [(0.55 – 0.0055 × RH %) × (1.8 × AT°C – 26)]. Since macro-climate elements at Meteorology observatory were recorded at 7:00 and 14:00 hours of the day, for the purpose of this analysis, records of 7.00 and 14.00 hrs of micro-climate (logger data) were used. The association and difference between macro- and micro-climatic parameters AT, RH and THI were analyzed using Pearson's correlation and paired 't' test. The effect of micro climate on seminal attributes of bulls were analyzed using repeated measures in GLM modeling of SPSS 26 software (IBM Pvt Ltd, Bengaluru, India). The effect of micro-climatic parameters were evaluated at weekly, fortnightly and seasonal level. Breed-wise fortnightly Mean ± SEs of semen quality traits and oxidative markers were calculated and Pearson's correlations were drawn with fortnightly mean climatic elements to explore association between them.

Results and Discussion

Environmental Findings

Total 8540 hourly observations of maximum, minimum and mean AT and RH of each logger (n=04; 8540 x 04 =34,160) were made during Jan 2018 to Dec 2018. Excluding error and missing values (n=740 paired values), daily micro-climatic parameters were calculated. The correlation between AT, RH and calculated THI from four data loggers hanged at different places were higher (r= 0.985 (AT), r=0.91 (RH), r=0.96 (THI); $p < 0.001$) with no significant difference between AT (p=0.137), RH (p=0.146) and THI (p=0.132). Therefore average of corresponding measurements of AT, RH and calculated THI of four loggers were used for further analyses. Coefficients of correlation between daily averages of AT, RH and THI measured at the bull shed and meteorological station were r= 0.981 (n=330; p=0.000), r= 0.897 (n=330; p=0.000) and r= 0.985 (n=330; p=0.000), respectively. Overall, AT was 1.20±0.14°C higher, RH was 5.42±1.27% lower and the THI was 0.50±0.23 higher at bull shed compared with meteorological station (p=0.000; Table 1).

The higher micro-climatic AT and THI in this study is in accordance of earlier reports (Schüller *et al.,* 2013; Shock *et al.,* 2016). Further these results suggest that microclimate within bull shed could be affected by confounding condition, ventilation, number of animals, position and orientation of housing, cleaning practices of housing and magnitude of interaction between these factors (Collier *et al.,* 2006; Schüller

Table 1: Mean (±SE) values of climatic parameters of the semen station (micro-climate) and meteorological station (macro-climate)

Parameters	*Paired Observations (n)*	*Micro (Mean ± SE)*	*Macro (Mean ± SE)*	*p-Value*
Ambient Temperature (°C)	330	28.59± 0.83	27.39± 0.88	0.000
Relative Humidity (%)	330	57.40± 2.32	62.82± 2.86	0.000
Temperature Humidity Index	330	76.97± 1.16	76.50± 1.26	0.001

et al., 2013; Shock *et al.,* 2016). The daily circadian hourly rhythm of AT, RH and THI in collection shed is documented in Figure 1 and 2. The maximum AT, RH and THI were observed at 16:00, 6:00 and 16:00 hours of the day, respectively. The minimum AT, RH and THI were found at 7:00, 16:00 and 7:00 hours of the day, respectively. The weekly mean micro- and macro-climatic parameters AT, RH and THI measured at meteorological station and bull shed are presented in Figure 3. Most studies (Suthar *et al.,* 2012; Shock *et al.,* 2016) and scientific reviews (Collier *et al.,* 2006; De Rensis *et al.,* 2015; Polsky and von Keyserlingk, 2017) suggested that THI above 72 causes stress and affects body temperature, production and reproduction function of dairy animals. In our study, 72.6 % of hourly observations of micro THI were above 72, which suggest that bulls in the study experienced maximum stress hours (Ravagnolo *et al.,* 2000; Polsky and von Keyserlingk, 2017).

Semen Parameters and Associations

Total 26 ejaculates of each bull of three Gir, Murrah and Surti breeds collected at bi-weekly interval during the study period were evaluated for semen quality and seminal plasma oxidative markers. The breed-wise seasonal means of seminal attributes and oxidative markers are presented in Table 2. The effect of environmental parameters on semen quality did not reflect serious effect of climate/month, except in July-August ($p < 0.05$). Similar trend was observed by Bhakat *et al.* (2011) in Sahiwal bulls. They have observed production of quality semen during rainy season. Similar to our findings, Verde *et al.* (2020) evaluated seasonal effect on the distribution of fertility-associated proteins in seminal plasma of six bulls of the American Brahman breed (*Bos indicus*) from Thailand. Results of their study did not reflect any effect of THI during winter, summer and rainy season and concluded that this might be due to acclimatization and/or adaptation of the bulls. Sharma *et al.* (2018) from Utter Pradesh, India also did not observe any impact of THI on ejaculate volume and pH of buffalo bulls semen between the THI groups, but THI >78 had impact on mass motility and progressive sperm motility ($p < 0.05$). Many investigators from India reported similar results using season in their study (Dhami *et al.,* 1998; Bhakat *et al.,* 2011).

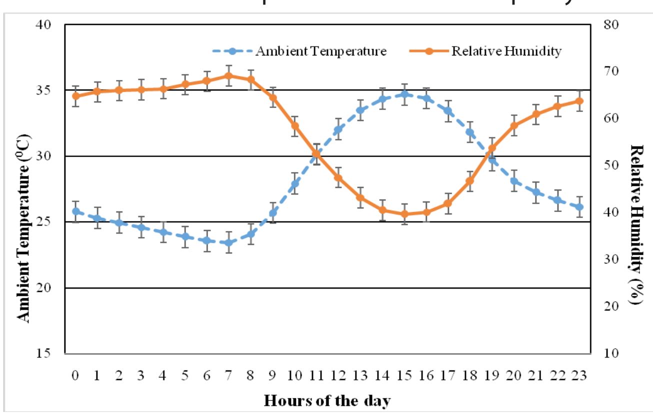

Fig. 1: Daily hourly rhythm of ambient temperature (AT °C) and relative humidity (%) measured at semen station (Micro-climate) using four Tinytag Plus II loggers

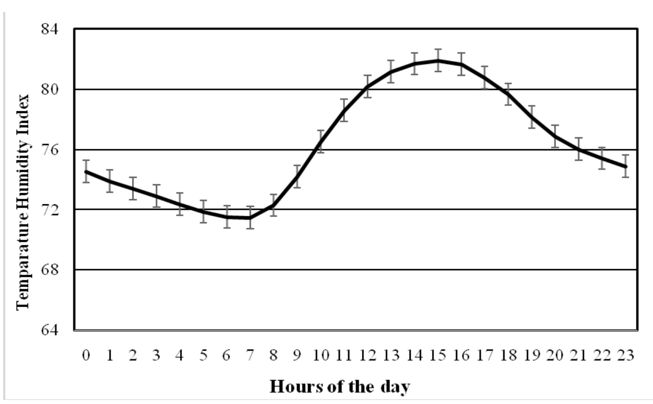

Fig. 2: Daily hourly rhythm of temperature humidity index (THI) measured at semen station using four Tinytag Plus II loggers

The report from north-west India (Soren *et al.,* 2016) show that the seasonal influence on oxidative markers such as glutathione peroxidase, glutathione reductase, malondialdehide and SOD was very much pronounced in Karanfries (HF X Sahiwal; n=05) bulls and other crosses. Nichi *et al.* (2006) in Nellore (*Bos indicus*; n=11) and Simmental (*Bos taurus*; n =16) bulls raised under tropical conditions of Brazil demonstrated that during summer stress the magnitude of response of oxidative markers was higher in Simmental bulls than Nellore bulls. This might be the reason in our study where limited association or effect was observed on oxidative markers of Murrah and Surti buffalo or Gir cattle bulls.

The coefficient of correlation between micro-climatic data and semen quality traits including oxidative markers for Gir, Surti and Murrah bulls are given in Tables 3 and 4, respectively. Our findings on coefficient of correlation between major seminal attributes and oxidative markers showed similar

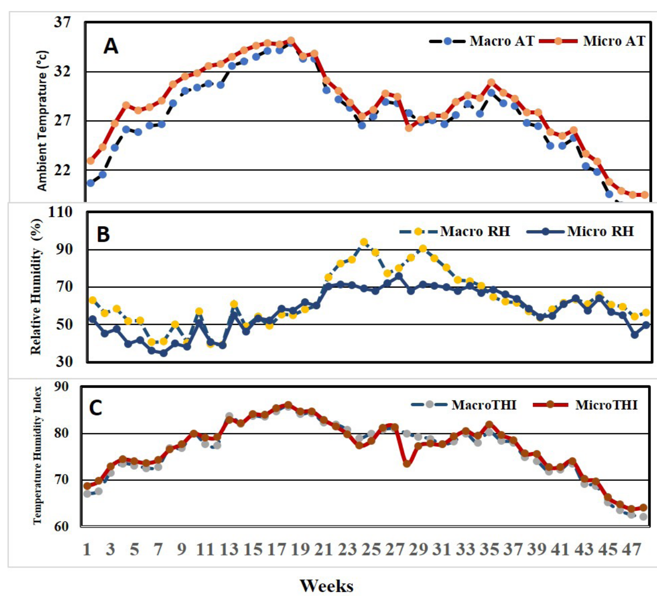

Fig. 3: Weekly ambient temperature (AT; A), relative humidity (RH %; B) and THI; C) measured at semen station (micro-climate) and meteorological station (macro-climate)

Table 2: Seasonal average semen quality and seminal plasma oxidative markers in Gir cattle, and Murrah and Surti buffalo bulls (Mean±SE)

Breed/ Species	*Season*	*EV*	*IM*	*SC*	*LS*	*AS*	*HOST*	*Catalase*	*MDA*	*SOD*	*GPX*
Gir Cattle (n =03)	Winter	6.28±0.26	76.51±1.15	1159.23±46.55	81.43±0.7	4.65±0.24	70.18±1.51	308.05±38.67	23.24±1.89	1.59±0.16	346.9±46.34
	Summer	6.42±0.22	74.83±1.88	1228.02±71.16	81.37±0.88	5.41±0.42	69.39±3.29	293.86±59.86	24.46±2.14	1.38±0.13	391.96±41.05
	Monsoon	7.06±0.26	74.58±2.31	1147.87±32.42	77.62±1.27	7.01±0.28	67.33±1.72	295.44±79.26	22.72±1.97	1.60±0.11	334.38±55.2
	Overall	6.59±0.25	75.31±1.78	1178.37±50.04	80.14±0.95	5.68±0.31	68.97±2.18	299.12±59.26	23.47±2.00	1.52±0.13	357.75±47.52
Murrah Buffalo (n =03)	Winter	3.77±0.26	79.09±1.39	1125.08±40.97	81.83±0.90	4.38±0.32	66.185±1.89	271.75±21.27	21.41±1.95	0.97±0.23	384.75±22.71
	Summer	3.42±0.19	75.07±1.24	1145.8±54.49	80.72±0.71	6.01±0.31	69.365±1.58	270.08±23.75	23.77±1.96	1.01±0.12	345.31±32.06
	Monsoon	4.58±0.31	74.58±2.47	1094.3±70.28	78.45±0.86	5.71±0.41	68.63±1.90	278.22±16.1	21.70±3.31	1.07±0.18	379.11±26.98
	Overall	3.92±0.25	76.24±1.70	1121.74±55.25	80.33±0.82	5.36±0.34	68.06±1.792	273.35±20.39	22.29±2.41	1.017±0.17	369.74±27.25
Surti Buffalo (n =03)	Winter	3.41±0.26	79.15±2.02	1092.91±18.66	83.03±1.21	4.25±0.32	71.04±1.06	296.45±22.17	19.40±0.78	1.18±0.15	451.53±41.10
	Summer	3.26±0.13	74.32±1.42	1210.36±35.61	81.63±1.1	4.51±0.50	74.31±1.20	298.23±30.71	20.2±1.25	0.99±0.18	451.27±23.59
	Monsoon	3.52±0.31	74.51±2.17	996.18±32.71	78.37±1.40	5.34±0.33	66.48±2.36	270.03±24.51	21.05±1.04	1.11±0.13	406.22±53.11
	Overall	3.40±0.23	75.99±1.87	1099.81±28.9	81.01±1.24	4.71±0.38	70.61±1.55	288.23±25.80	20.22±1.02	1.09±0.15	436.36±39.26

EV=ejaculate volume (ml); IM=initial motility (IM); SC=sperm concentration (million/ml); LS=live sperm (%); AS=abnormal sperm (%); HOST= hypo-osmotic reactive sperm (%); Catalase (U/ml) MAD=malondialdehide (µmol/ml); SOD=superoxide dismutase (µmol/ml); GPx= glutathione peroxidase (mmol/min/ml)Note: None of the attributes showed significant seasonal influence in any of the breed ($p > 0.05$).

Table 3: Pearson's correlations (r) amongst physical attributes, oxidative markers of Gir bulls' semen and macro-micro-climatic data

	IM	*SC*	*LS*	*AS*	*HOST*	*Catalase*	*MDA*	*SOD*	*GPX*	*MicAT*	*MicRH*	*MicTHI*
EV	-0.18	0.18	-0.46*	0.58**	-0.29	0.24	-0.17	-0.18	-0.47*	0.20	0.18	0.23
IM	1	-0.21	0.30	-0.41*	0.55**	0.55**	-0.46*	-0.13	0.12	-0.32	-0.11	-0.20
SC		1	0.08	0.28	0.19	-0.11	-0.03	-0.15	0.40*	-0.39*	0.02	-0.10
LS			1	-0.49*	0.57**	0.39*	0.25	-0.18	0.44*	-0.65**	-0.18	-0.35
AS				1	-0.27	0.03	-0.17	0.32	-0.11	0.37	0.22	0.31
HOST					1	0.73**	-0.19	-0.43*	0.55**	-0.71**	-0.33	-0.56**
Catalase						1	-0.37	-0.33	0.14	-0.51**	-0.20	-0.36
MAD							1	-0.11	0.14	-0.02	0.18	0.16
SOD								1	-0.27	0.43*	-0.29	-0.10
GPX									1	-0.68**	0.32	0.07
MicAT										1	0.08	0.38
MicRH											1	0.95**

EV=ejaculate volume; IM=initial motility; SC=sperm concentration; LS=live sperm; AS=abnormal sperm; HOST= hypo-osmotic reactive sperm; MAD=malondialdehide; SOD=superoxide dismutase; GPx= glutathione peroxidase. MicAT= Micro-climatic Ambient Temperature (°C), MicRH= Micro-climatic Relative Humidity (%), MicTHI= Micro-climatic Temperature Humidity Index; *$p < 0.05$; **$p < 0.001$.

trend as reported by Shelke and Dhami (2001), Pathak *et al.* (2018) and Patel *et al.* (2019) in bovines.

In Gir bulls, the coefficients of correlation evaluated (Table 3), showed that SOD had positive correlation and sperm concentration, live sperm, HOS reactive sperm, catalase and GPx had negative correlation with micro-AT ($p < 0.05$). No seminal parameters or oxidative markers had correlation with micro-RH ($p > 0.05$). Only HOS reactive sperm had negative correlation with micro-THI ($p < 0.05$). In Surti bulls, the coefficient of correlation evaluated (Table 4), showed that ejaculate volume and abnormal sperm had positive correlation and sperm concentration, HOS reactive sperm and catalase had negative correlation with micro-AT ($p < 0.05$). Initial motility and SOD had negative correlation and MAD had positive correlation with micro-RH ($p < 0.05$). While initial motility, live sperm and SOD had negative correlation and MAD had positive correlation with micro-THI ($p < 0.05$). In Murrah bulls, the coefficients of correlations (Table 5) showed that initial motility and live sperm had negative correlation, while ejaculate volume and catalase demonstrated positive correlation with micro-AT ($p < 0.05$). Initial motility and GPx had negative correlation and HOS reactive sperm and MAD had positive correlation with micro-RH ($p < 0.05$), while HOS reactive sperm and MAD had negative correlation and initial motility, live sperm and GPx had positive correlation with micro-THI ($p < 0.05$).

Some of seminal parameters and oxidative markers of all three breeds had demonstrated correlation with micro-climatic parameters ($p < 0.05$), particularly in Surti and Murrah buffalo bulls. These results corroborated with past studies on buffalo bulls (Sharma *et al.,* 2018) and cattle bulls (Nichi *et al.,* 2006). This might be due to species difference (cattle vs buffalo) or origin of Gir breed is from hot humid tract near Gir forest of Gujarat. Therefore this breed developed good adaptation to this condition. While Surti breed is from tract having hot tropical condition of central Gujarat of India. Similarly Murrah breed of buffalo is from hot tropical climate of north India. This might be reason why buffalo breeds in our study demonstrated more correlations with micro-climate. Interestingly in our study the coefficients of correlations were achieved, but the effect of season on seminal attributes or oxidative markers was not revealed. This might be attributed to indigenous species and breeds which shows better acclimatization and adaptation to heat stress. Having wrote that limited number of bulls in the study constraint the impact of this statement, however, Henson (2004, 2009) in his reviews very well documented the phenomenon of acclimatization and adaptation of zebu breeds. The results of present study warrant further investigation using more number of bulls, including their semen freezability and fertility.

Conclusions

The results suggest that macro- and micro-climatic AT (r=0.982), RH (r=0.897) and THI (r=0.985) were highly

Table 4: Pearson's correlations (r) amongst physical attributes and oxidative markers of Surti and Murrah buffalo semen and macro-micro-climatic data

	EV	*IM*	*SC*	*LS*	*AS*	*HOST*	*Catalase*	*MAD*	*SOD*	*GPX*	*MicAT*	*MicRH*	*MicTHI*
						Correlations: Surti buffalo bulls							
EV	1	-0.15	-0.03	-0.23	0.59**	-0.14	0.05	0.07	0.14	0.27	0.59**	0.18	0.33
IM	-0.57**	1	0.23	0.68**	-0.41*	0.18	-0.32	-0.22	-0.18	0.32	-0.07	-0.55**	-0.55**
SC	0.10	-0.35	1	0.28	-0.16	0.20	0.25	-0.23	0.16	0.49*	-0.41*	0.18	0.01
LS	-0.77**	0.82**	-0.23	1	-0.40*	0.27	-0.05	-0.12	-0.20	0.45*	-0.37	-0.36	-0.47*
AS	0.13	-0.58**	0.24	-0.52**	1	-0.55**	-0.20	0.05	0.21	-0.20	0.71**	0.20	0.38
HOST	-0.38	0.03	-0.03	0.21	-0.09	1	0.18	-0.31	-0.43	0.62**	-0.40*	0.31	0.16
Catalase	0.49*	-0.27	-0.21	-0.38	-0.27	-0.28	1	-0.56**	0.73**	0.43*	-0.59**	-0.16	-0.33
MAD	-0.34	-0.21	-0.07	0.17	-0.17	0.66**	0.10	1	-0.55	-0.53**	0.27	0.43*	0.51**
SOD	0.44*	-0.15	-0.42*	-0.17	-0.19	0.23	0.17	0.29	1	0.08	-0.21	-0.50*	-0.53**
GPX	0.27	-0.06	0.35	-0.26	0.25	-0.65**	-0.06	-0.80**	-0.58*	1	-0.33	-0.01	-0.14
MicAT	0.79**	-0.46*	0.07	-0.57**	0.13	-0.26	0.40*	-0.24	0.31	0.32	1	0.08	0.38
MicRH	0.06	-0.65**	0.05	-0.29	0.35	0.57**	0.17	0.67**	0.24	-0.52**	0.08	1	0.95**
MicTHI	0.30	-0.72**	0.01	-0.42*	0.33	0.46*	0.28	0.55**	0.38	-0.42*	0.38	0.95**	1
						Correlations: Murrah buffalo bulls							

EV=ejaculate volume; IM=initial motility; SC=sperm concentration; LS=live sperm; AS=abnormal sperm; HOST= hypo-osmotic reactive sperm; MAD=malondialdehide; SOD=superoxide dismutase; GPx= glutathione peroxidase; *$p < 0.05$; **$p < 0.001$.

associated. Overall, micro-AT was 1.20±0.14°C higher, micro-RH was 5.42±1.27% lower and the micro-THI was 0.50±0.23 higher at bull shed compared with observatory data. Climatic AT, RH and THI demonstrated circadian rhythm during the day. During the year, 72.6 % of THI hourly measurements demonstrated stress above threshold 72. The micro-climatic parameters demonstrated significant association with seminal attributes like sperm concentration, live sperm, HOS reactive sperm, and catalase in seminal plasma of Gir bulls, and with ejaculate volume, initial motility, catalase, MAD and SOD of buffalo bulls. Overall seminal attributes and oxidative markers demonstrated an association with environmental parameters in all three breeds. Further mostly these parameters showed significant effect during June to August months of the year when humidity and temperature remained very high. Thus, the study suggest that indigenous Gir cattle and Murrah and Surti buffalo bulls demonstrated acclimatization and adaptation to the local tropical climate; however further evaluation of semen freezability and fertility on more number of bulls is warranted.

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