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Comparative study of seasonal variation in semen characteristics of buffalo bull

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Abstract
Heat stress is a well-known problem causing huge economic losses to the buffalo breeders as well as dairy industry. Heat stress has a direct effect on breeding efficiency of both male and female buffalo and reduces the performance of buffalo bulls. Identification of the factors which affect the spermatic production and the quality of the semen has a major importance in improvement of bull fertility. A total of 42 ejaculates from 2 buffalo bulls were collected twice a week through AV (artificial vagina) method for 6 months at Semen Production Centre, department of Veterinary Gynaecology and Obstetrics, Pantnagar India, and analyzed using least squares analysis to quantify the effect of temperature humidity index (THI) on various semen quality parameters. The THI was divided into 3 groups, i.e. Group I (64 to 72, n=10), Group II (72 to 78, n=13) and group III (78 to 84, n=18). THI variations had no significant effect on Hypo-osmotic swelling test (76.33±3.38, 73.33±3.44 and 72.00±4.76) and Methylene Blue reduction test (7.60±0.79, 6.93±1.01 and 7.47±0.95) but had a significant (P<0.05) effect on percent live sperm (83.00±1.07^{b}, 80.67±2.18^{ab} and 77.67±1.98^{a}) and Concentration of sperm (973.33±1.00^{b}, 935.00±0.90^{ab}and 900.00±1.50^{a}) The results of this study indicated that the live sperms and concentration of sperms decreases significantly (P<0.05) with the increase in THI. The value of percent live sperm, concentration and HOST reactive sperms was found highest at mean THI value of 68.42 (Group 1). Thus, it may be concluded that the hot-humid season with Higher THI adversely affect the various seminal attributes of buffalo bull.

Keywords: Season, temperature humidity index, semen quality parameters, buffalo bull

1. Introduction
India is producing about 66 million straws, which is covering only 20-25% breedable bovine population. To achieve the national target of covering 50% breedable bovine population there is need to produce 140 million [1] of frozen semen straws. Therefore, semen stations throughout the India are giving emphasis on production of required number of frozen semen doses, but there are various factors which influence the quality and required quantity of semen. The central monitoring unit of government of India is also periodically evaluating the performance for producing good quality germplasm. To achieve the quality criteria we need to intervene at the time of semen collection and managemental conditions present, which are the important aspects for quality semen production. In recent years, studies carried out emphasize the importance intensive study on managemental conditions particularly temperature and humidity in relation to animal productivity. As a result, these studies have grown newer concepts of management- feeding and handling of animals and designs for housing or shelter for the livestock as well as the lay-out of the immediate surrounding to the animal houses. In the tropical climate heat stress is largely responsible for the low animal productivity. Tropical countries like India, where ambient temperature remains above the thermo-neutral zone of the farm animals for a large part of the year, Heat stress is mainly responsible for the reduced performance of bovines during the period of year which is hot and humid. Since productivity is primarily determined by the extent of the utilization of the available natural resources as well as the direct effects of climatic components on the physiology of the animals, it is necessary to ascertain the detrimental influences of climatic condition particularly temperature and humidity on animal productivity. Investigations carried out under carefully controlled conditions have shown that both the magnitude and duration of stress induced by the adverse environmental condition influences the animal's physiological reactions and production, in the experimental location. The identification of the environmental factors which may affect sperm production and the quality of the semen has a major importance in improvement of bull fertility. Seasonal effects on semen quality have been observed in several species including

rams [2], Boer and Angora goats and rams [3], and Sahiwal and Brahamn crossbred bulls [4]. However, contradictory results were observed between different investigations concerning the effects of different seasons on semen quantity and quality. Most previous investigations indicated slightly higher semen quantity and quality during hot and humid periods during which mean temperature and humidity are higher than during the colder months [5]. The knowledge of trend of seasonal influence on semen characteristics would help to know the requirement of bulls to meet the demand of frozen semen and to provide any suitable additional managerial requirements time to time. Hence, the present study was undertaken to investigate the effect of seasons in reference to ambient temperature and relative humidity on semen characteristics of the cattle bull.

2. Materials and Methods

A total of 41 ejaculates from 2 Murrah buffalo bulls (5 to 6 years of age and 500 to 750 kg body weight) maintained at Semen Production Centre, Department of Veterinary Gynaecology and Obstetrics, Pantnagar, India. The bulls were healthy, free from diseases, sexually mature, good libido and clinically normal. Semen was collected in the morning twice a week from the bull using sterilized artificial vagina (temp 42-45 °C), vagina during different months of year i.e. April to October 2015 using dummy bull. Soon after collection, each ejaculate was placed in a water bath at 30-35 °C and standard laboratory evaluation for semen was recorded. The THI was divided into 3 groups. First group (n=10) having the mean THI range of 64 -72, 2nd group (n=13) having the THI range of 72-78 and the 3rd group (n=19)78-84.

Quality of semen was assessed for volume and microscopic tests such as, progressive motility, concentration, HOST, and MBRT. Soon after collection the volume of whole ejaculate was measured. Colour and consistency were assessed by direct visualization. The pH was measured by Whitman's pH indicator paper. Motility of spermatozoa was estimated within 10-15 min from the time of semen collection. Progressive or individual motility was examined in semen diluted with one or two drops of citrate buffer, on a glass slide covered with slip, using high power microscope. The percentage of individual spermatozoa displaying progressive motility across the field was estimated and recorded. The temperature and relative humidity of respective days was taken from meteorological Department located nearby. The recorded data were subjected to statistical analysis using Least squares means model and ANOVA using LSML-91 software package, Walter Harvey.

3. Results

Table 1. Presents the effect of THI on various seminal attributes of Murrah buffalo bulls (Fig 1-4).

THI variations had no significant effect on percent HOST reactive sperms and MBRT in Murrah buffalo bulls, but it had a significant (P<0.05) effect on percent live sperms and concentration of sperms. The results are presented in table 1.

The present data clearly indicated that there was significant (P<0.01) decrease in percent live sperms in group 3rd (77.67±1.98^{a}) when THI increased to mean value of 80.54compared to group 1st (83.00±1.07^{b}) however, non-significant difference was found between group 3rd (77.67±1.98^{a})and group 2nd (80.67±2.18^{ab}). Percent live sperms varied non-significantly between group 1st (83.00±1.07^{b}) and 2nd (80.67±2.18^{ab}) when THI increased from mean value of 68.42 to 75.95. The highest and lowest percentage of live sperms was estimated to be 83.00±1.07 and 77.67±1.98^{a} % at mean THI of 68.42 and 80.54 respectively and clearly indicated the decrease in percent live sperms in ejaculate of buffalo bull with the increase in THI.

The results revealed that the sperm concentration per ml (SPC) varied significantly (P<0.01) among 3 groups of THI being maximum at mean THI value of 68.42 (973.33±1.00) and lowest at mean THI value of 80.54 (900.00±1.50). Significant decrease in concentration of sperms was found in group 3rd (900.00±1.50) compared to group 1st (973.33±1.0) however it decreased non-significantly with group 2nd (935.00±0.90).

The results revealed that there was non-significant decrease in percent HOST reactive sperms (76.33±3.38, 73.33±3.44 and 72.00±4.76) when mean value of THI is increased from 68.4 to 75.95 and then to 80.54, respectively. The highest (76.33±3.38) and lowest (72.00±4.76) percent of HOST reactive sperms was observed at THI of 68.42 and 80.54 respectively.

The results of MBRT indicates non-significant change in MBRT values with the change in THI among all 3 groups with highest and lowest value being found at mean THI value of 80.54 and 68.42, respectively.

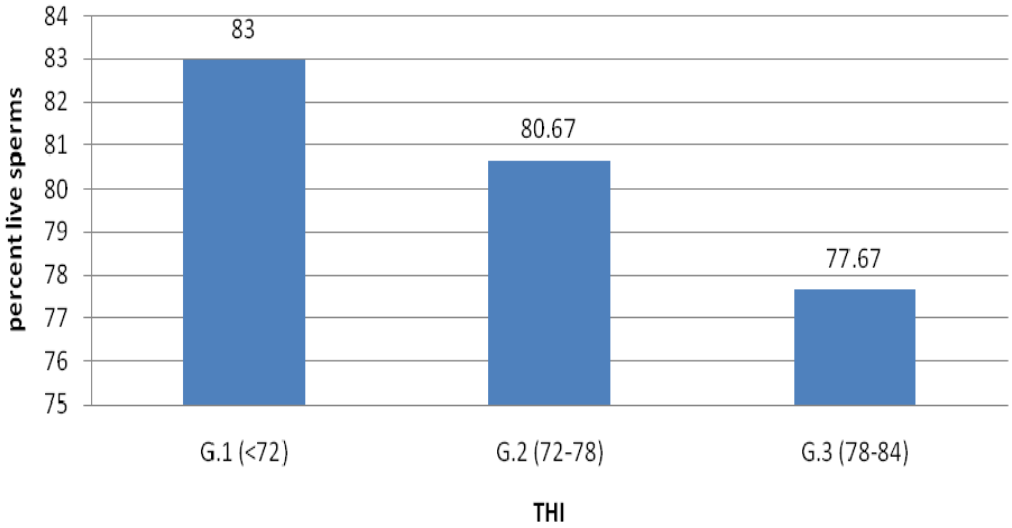

Fig 1: Effect of THI on percent live sperms

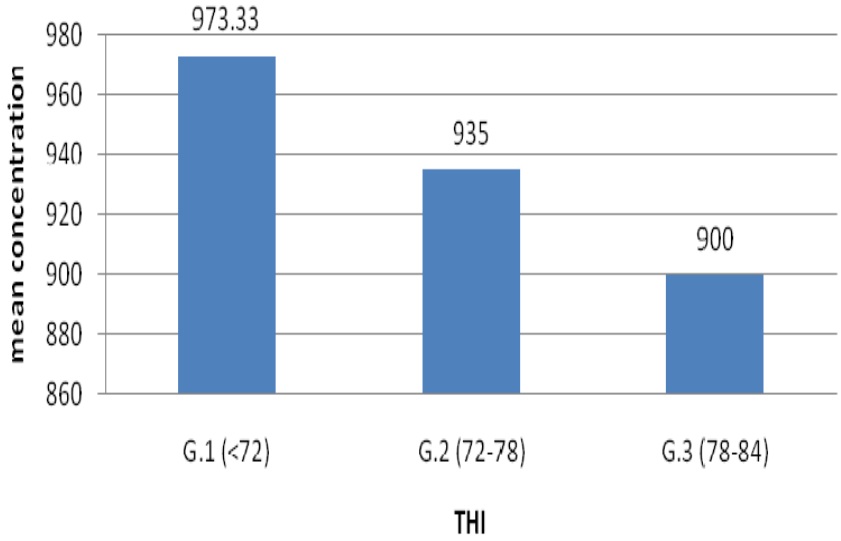

Fig 2: Effect of THI on mean concentration of sperms (million/ml)

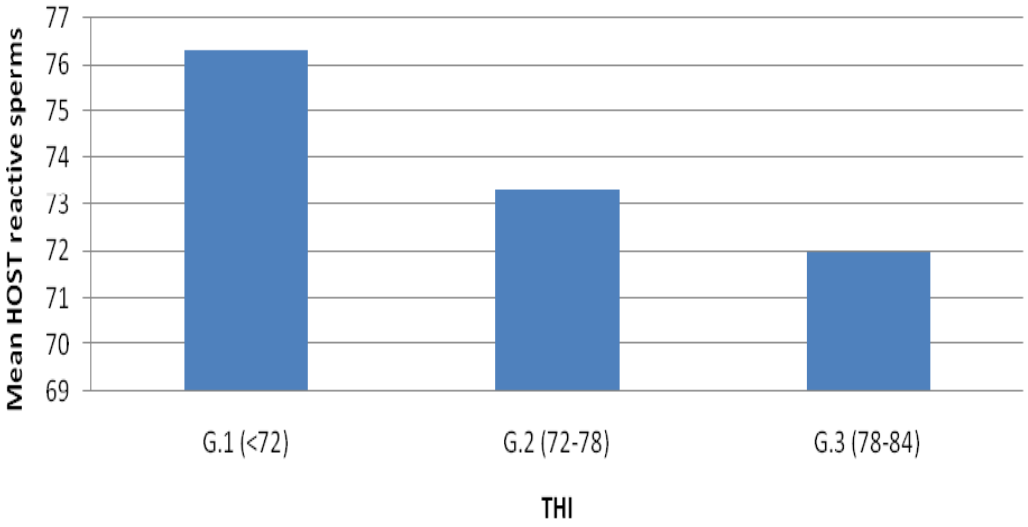

Fig 3: Effect of THI on HOST of sperm

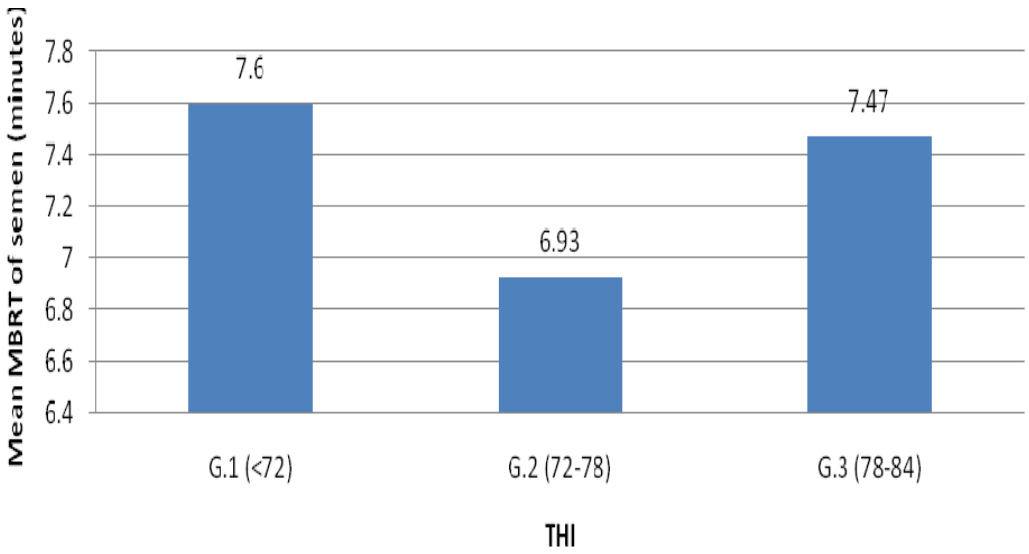

Fig 4: Effect of THI on MBRT of sperm

Table 1: Means bearing different alphabets as superscripts differ significantly row-wise (abP<0.05)

Parameters	Group-I (n=10)	Group-II (n=13)	Group-III (n=19)
% live sperm	83.00±1.07^{b}	80.67±2.18^{ab}	77.67±1.98^{a}
Conc. of sperm (million/ml)	973.33±1.00^{b}	935.00±0.90^{ab}	900.00±1.50^{a}
HOST	76.33±3.38	73.33±3.44	72.00±4.76
MBRT(min)	7.60±0.79	6.93±1.01	7.47±0.95

4. Discussion

Efficient semen collection from breeding bulls is an art and an important activity of frozen semen production centre. The management of entire process from bull rearing and training to semen collection is critical, for which the management conditions particularly temperature and humidity play a vital role in efficient and quality semen production. Ambient temperature and relative humidity vary from day to day and ameliorate during the night. Scrotal circumference, testicular consistency, tone, size and weight are decrease in hot summer in the sub tropics than those of the same breeds reared under temperate environmental conditions [6]. This reduction in testicular measurements might be due to degeneration in the germinal epithelium resulting in decrease in quality of semen production [7].

According to the results obtained in this study, the % live sperms and concentration of sperms decreased significantly with the increase in THI value and the value of these two parameters was found highest at mean THI value of 68.42. There was non-significant change in percent HOST reactive sperms and MBRT of semen with the increase in THI value.

Similar to our study, sperm concentration per ml (SPC) varied significantly (P<0.01) among seasons being maximum during winter when THI was on lower side followed by rainy and summer season [8, 9] whereas highest sperm concentration was obtained during rainy season [10] and [11]. In Egyptian buffalo bulls it was higher during spring [12]. Also the lowest sperm concentration was recorded during summer, when THI index was 65.39, and THI-week index was 67.30. In agreement to our results lowered sperm concentration, HOST % and total noneosinophilic sperm output during hot humid (THI is on higher side) was observed as compared to summer season [13] and spring season in Karan Fries bulls [14]. Good quality of semen was observed during spring season where the environment temperature was approximately 25 °C. Sperm production and quality (ejaculate volume, sperm concentration and total sperm number) and percentage of normal sperm cells) decreased during the hot season in *B. indicus* bulls [15, 16]. In a study, Reproductive and physiological responses under varying artificial incandescent illumination, temperature, and humidity conditions was studied in bulls and significant differences (P<0.01) was observed in percent HOST reactive and methylene blue reduction time (modified). However, concentration of spermatozoa showed non-significant differences. In agreement with our results, Non-significant relationship of THI with percent HOST reactive spermatozoa was also observed. HOST % was related significantly and positively to the sperm motility. However [14], documented significant (P<0.05) decrease in percent HOST reactive sperms during hot humid season during which THI is on higher side as compared to spring season. In our study, no significant change was observed in MBRT time which could be because of dependency of MBRT on the metabolic activity of spermatozoa not on the concentration. Summer conditions consisting of above normal ambient temperature, relative humidity, and solar radiation increase the thermal load on animal and causes significant decrease in production potential and may also be detrimental to health of animal. The detrimental effect of high environmental temperature on semen quality and male fertility may be attributed to the hypothalamo–hypophyseal pathways or to a direct effect of temperature on testicular and epididymal function.

Significant (P<0.05) seasonal influence on motility and sperm concentration in Murrah buffalo bulls was observed [6] in a study where 4446 ejaculates were observed over a period of 7 years to know the seasonal influence on various seminal attributes, in Murrah bulls. Sperm concentration was highest in summer whereas, non-significant effect of season on progressive motility was observed [17]. In contrast to our study, non-significant seasonal variation in sperm concentration was obtained in Nili- Ravi buffalo bulls [18], in Murrah and Surti [19] and in Holstein (*B. taurus*) bulls [4] and Frieswal bulls [20]. Similarly, effect of season on semen quality parameters of swamp buffalo was observed and sperm concentration, total sperm number and initial sperm motility did not differ between seasons, whereas PMI and the relative proportion of morphologically normal spermatozoa were highest in summer and lowest in winter [21]. Holstein bulls produced more sperm (higher sperm concentration and total sperm number) with greater motility during the winter and the spring (low THI) compared to summer [22]. Ambient temperature and humidity did not significantly affect sperm production and semen quality.

In general, of all seasons, summer exerts comparatively more adverse effect on the overall quality of semen. It effects the normal reproduction process multidimensionally, like by reducing feed intake, by inhibiting the release or response to important reproduction hormones that is, GnRH, FSH and LH. During summer, thyroxin secretion declines leading to reduced intake of feed by the animal, subsequent metabolism [23] and is responsible for reducing production of sperm [18]. Due to extreme heat stress bulls get physically exhausted and their reduced eagerness might result in higher reaction time and subsequently total time for successful ejaculation also increase, thus having an ultimate effect on production of sperms [8].

5. Conclusion

Hot-dry or summer season during which THI values are found to be towards higher side (THI 78-84) affect the various biophysical characteristics of semen in buffalo bulls. Elevation of environmental temperature during summer affects male reproductive functions deleteriously. Such deleterious phenomenon leads to testicular degeneration, sperm mortality and increases the sperm abnormality. Progressive motility and concentration gets decreased significantly at the THI value of 78-84 whereas percent HOST and MBRT value were non- significantly affected. THI values in normal range (winter) was the most favorable season for good quality semen production. Specific consideration to the surrounding environmental conditions

particularly temperature and humidity should be paid to minimize their negative influence directly or indirectly on semen production and other related physiological functions of the breeding bulls

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