INFLUENCE OF THERMAL STRESS ON POST-PARTUM REPRODUCTIVE PERFORMANCE OF CROSSBRED DAIRY COWS

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THESIS

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2020

DECLARATION

I hereby declare that this thesis, entitled **"Influence of thermal stress on post-partum reproductive performance of crossbred dairy cows"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Dr. C. Ibraheem Kutty

DEDICATION

To all those who

1. **understand the value of time and act accordingly**

2. **recognize own responsibilities more importantly than privileges**

3. **considers nature’s way and principles while intervening biological processes**

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INTRODUCTION

1. INTRODUCTION

India is basically an agriculturebased economy and livestock sector contribute 25.6 per cent of the agricultural GDP (DAHD, 2019).Reproductive rate is one of the major determinants of profitability from any type of animal production enterprise. Consequent to genetic alterations and improvement of management standards, considerable increase in milk production of dairy cattle has been recorded worldwide. At the same time, fertility of these animals started declining, marginalizing the benefits of hike in milk production.Various forms of stress factors, which interfere with hormonal milieu of the animal has been attributed causative for such a decline in fertility.

Essence of reproduction is propagation of the species and demands utilization of bodily reserves, time and effort; and also need to be regulated in anticipation of the congenial environment for survival of the new born. Accordingly, whenever there are adversities of the environment, current or anticipated, reproductive processes are postponed for the want of better nutrition and favourable climate. This is made possible by the regulation of reproduction by a very low threshold for adverse climatic factors so that underlying processes gets affected well in advance than any other systems. Also the functional impairments persist forsome more time even after reversal of the climatic adversities and regaining normality of other systems (Torres-Junior *et al.,* 2008).

Influence of adverse climate on reproductive system not only causes postponement of initiating new processes but also leads to the arrest of already initiated steps. Consequent lowering of reproductive performance seriously affects the productivity in long term unless adequate preventive or ameliorative measures are taken up well in time and such interventions necessitate early detection measures or suitable predictions.

Considering the increasing demand for milk, local cattle of Kerala state were converted into crossbreds of high producing exotic dairy breeds like Holstein Fresian (HF), Jersey and Brown Swiss (BS). Currently more than 95 per cent of the cattle in the state are crossbreds having better potential for milk production. However, even after continued breeding and management efforts over four decades, milk production of crossbred cattle has increased three to four folds only, with an alarming hike of production cost. Prominent reason forthe slow pace of productivity hike appears to be the increased susceptibility of these animals to various forms of stress and is manifested by the phenomenon of not attaining the performance level, expected based on the genetic potential,increased incidence of diseases and lowered reproductive rate (Kumar and Rao, 2013).

Climate change has become one among the major threats for enhancement of animal productivity especially at the tropical regions with high ambient temperature (AbT). An increase of atmospheric temperature (AtT) upto 0.2 degree Celsius (^{o}C) per decade, reaching 1.5^{o}C above the level of pre-industrial period, between 2030 and 2050 has been projected by IPCC (2018). Hot humid climate prevailing in Kerala has already found hostile to increase the productivity of dairy cattle. Extended rainy season and stretched out sea shore contributes high relative humidity (RH) during most part of the year.Thus, consistently high RHtogether withmoderately high AbTcaused by geographical proximity to the equator makes the climateof Kerala highly adverse for animal welfare throughout the year, compared to other regions of the country.

Paradoxically, while the vulnerability of animals to stress factors are increasing, the management situation is becoming more and more adverse, contributed by urbanization and the climate change. Such a divergent transformation not only threatens the survival of productive animals but in those able to tolerate the hostility, impairments of reproductionis highly inevitable. Hence, various stress alleviation measures to restore the productivity are being incorporated in the management with variable effectiveness(Prasad 2014). However, such measuresare of little benefit for restoration of impaired fertility especially because of the prolonged consequences of thermal stress (TS) on reproductive processes.

Thus, it is essential to have a precise understanding of the mechanism of fertility impairments consequent to TS. Also,physiologicalindicators for an imminent fertility reduction caused by TS and the stage to intervene for effective prevention of the same are yet to be established.Hence, the study was carried out with the following objectives

1. To elucidate the influence of thermal stresson post-partumreproductive performance of crossbred dairy cows
2. Identify suitable physiological indicators for assessing the magnitude of thermal stress on fertility of crossbred cows.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. THERMAL STRESS IN CATTLE

In general,mammals belong to homeotherms, capable of regulating their body temperature (BT) within a narrow range irrespective of the surrounding temperature. However, even the thermoregulatory mechanisms were found to cause strain on the body systems especially when the margin of regulation needed was wider (Kadzere *et al*., 2002). Metabolic processes has been found to generate heat and elevation of the same beyond the normal limit prescribed for the species necessitated dissipation of the excess heat into the surroundings (Mishra and Palai, 2014). The effectiveness of the heat dissipation mechanism was determined by the rate of heat generation inside the body and temperature difference between the body and the surroundings. Accordingly, wheneversurrounding temperature increased nearer to or beyond BT, effectiveness of heat dissipation was reduced and resulted in disruption of the homeostasis with consequences such as lowered production, impairment of reproduction and even threatening the existence of the animals depending upon the severity of temperature elevation (Mader *et al.,* 2006).

Two of the recent phenomena such as increased milk production of dairy animals with resultant elevation of internal heat production and global warming that have caused elevation of the surrounding temperature; both have become significant contributors of TS (Schuller *et al.,* 2014). Thus, dairy animals especially cattle have become highly prone to TS (Hansen, 2015) and various consequences of the same have started to interfere the productivity of these animals (Das *et al*., 2016). Earlier, TS was considered as a phenomenon restricted to tropical regions, but recently, global warming has widened the limit for the occurrence of TS as a major issue even in temperate countries (Polsky and Von-Keyserlingk, 2017). Similarly, besides dairy cattle, beef cattle and even other species including wild animals have been affected by TS manifesting consequences like behavioural alterations, altered growth pattern and impaired reproduction (Hansen, 2009 and Hansen, 2015)

The term TS covers various external forces acting on an animal leading to the elevation of its BT to such a level that evokes some undesirable physiological response (Dikmen *et al*., 2008). Excessive elevation of BT caused by inflow of thermal energy from the surrounding and retention of internally generated heat beyond the level for maintenance of normal BT often caused disruption of the quality of life and productivity(Das *et al*., 2016).

Hence, continuation of living under such conditions necessitated various adaptive mechanisms intended to minimizeinternal heat generation and enhancement of net outward flow of heat so that comfort of living gets improved (Polsky and Von-Keyserlingk, 2017).

Thermal stress was often considered as a problem affecting lactating dairy cows, owing to their high rate of metabolism and non-lactating animals such as dry cows, heifers and beef cattle were less affected (Badinga *et al*., 1985; Dikmen and Hansen*et al*., 2009). Increased milk production of lactating animals wasaccompanied by high metabolic rate necessitated by excessive drainage of nutrients, increased intake of water and feed to compensate the loss, replenishment demands of the transition phase and additional needs for supporting pregnancy and/or growth demanded by the management situations(Hansen, 2009). Large amount of ingesta was found to elevate the internal heat production consequent to digestion(including rumen fermentation) and associated metabolic processes, and thus increased the susceptibility of lactating animals to TS especially when AtTexceeded beyond the normal limits (Hansen, 2015).

Thermal stress not only reduced the quantity of daily milk production, but adversely affected the milk composition as well as lactation length (Rhoads *et al*., 2009; Guo *et al*., 2018). Total annual loss of milk production contributed by TS alone in India was estimated in the year 2008 as 1.8 million tones which formed approximately 2per cent of the total milk production of the country valued Rs. 2661.62 crores per year (Upadhyay *et al*., 2009). Further, the negative impact of global warming on total milk production in India was projected to reach 3.2 million tons by the year 2020 and is also expected to exceed 15 million tons by the year 2050 (Das *et al*., 2016).

The initial response of animalssubjected to elevated thermal weather was enhanced breathing and heart rate so that heat dissipation was improved (Hansen, 2009). Subsequently, sweating and open mouth breathing were initiated to enhance the evaporative heat loss. Elevation of BT directly affected the feed intake,causing reduction of milk yield, growth rate, fertility and even death in extreme cases (Allen *et al*., 2009). Long term persistence of TS resulted in suppression of the immune mechanism thereby making the animal susceptible to various diseases (Kadzere *et al*., 2002). Disruption of the endocrine system affected metabolic processes andcaused major reduction of the reproductive performance (De-Rensis *et al*., 2015). Thus, sustainable dairy farming has become major challenge in the context of ongoing climate change scenario(Hansen, 2015; Das *et al*., 2016).

2.2. CLIMATIC VARIABLES INFLUENCING THERMAL STRESS

Thermal comfort of animals are regulated by a balance between heat generation and dissipation so that BT is maintained within the normal range prescribed for the species, utilizing minimum internal resources and at the same time maintaining all the normal physiological processes unaffected. Various climatic variables were found to challenge the thermoregulatory mechanism of the body and major ones were AbT, RH and thermal radiations (Marai *et al.*, 2008). Dairy cows were generating lot of metabolic heat as the result of increased secretion of milk and positive heat loss formed the major component of thermoregulation through dissipation of the excess heat(El-Tarabany and El-Tarabany, 2015). Thus, climatic variables that caused elevation of the surrounding temperature beyond the thermo-neutral range produced TS by modifying the means of heat dissipation from passive to active transfer. However, such a transition necessitated physical effort, expenditure of energy and disturbances of various physiological processes (Rashamol *et al.,* 2018).

In tropical and sub-tropical regions, high AbTformed the major climatic variable contributing TS (Marai *et al.*, 2008) and its influence was aggravated by accompanying ambient humidity (Allen *et al.,* 2009; Kumari and Pampana, 2015). These two variables acted together to produce impairments of the functioning of various tissues or organs of the body and affected normal maintenance, growth, production and reproduction of the animals exposed(Krishnan *et al*., 2017). Elevation of AbTstimulated metabolic processes that lead to further accumulation of metabolic heat unless heat dissipation mechanisms were also activated (Singh and Singh, 2005)**.** In this respect, elevation of humidity interfered the heat transfer into the surrounding by minimizing the chances of evaporation and aggravated the situation making it more stressful

Temperature humidity index (THI) forms a composite measure that simultaneously considers AbT and RH to understand the thermal load contributed by these two variables (Allen *et al.,* 2009). The THI values were calculated using formula and the obtained values were broadly categorized into two TS levels demarcated by an index value of up to 72 and above 72 (ranging from 75 °F or 23.9 °C and 65per cent RH to 90 °F or 32.2 °C and 0per cent RH) reported by Armstrong (1994). THI 72 formed the threshold limit for better milk yield and reproductive performance in tropical and sub-tropical climate (Armstrong, 1994; Krishnan *et al*., 2017). However, recent observations in temperate countries have indicated the need for lowering the threshold limit to THI 68 for ensuring better performance and welfare of high producing dairy cattle (Krishnan *et al*., 2017).

Besides high AbT, other climatic factors were also involved in the regulation of physiological processes especially reproduction in synchrony with the season (Marai *et al*., 2008). Such a regulation was more evident in temperate countries, wherein declining fertility was evidenced even though AbTand THI were within the comfortable range (De-Rensis*et al.,* 2017). One of the major climatic factors other than AbT is photoperiod even though a clear distinction of the effect of day length and AbTwas difficult since both these variables were inter-dependent and controlled by the duration of sunshine (Orihuela, 2000; Kilgoura *et al*., 2012). Other climatic factors contributing TS in animals included intensity and hours of solar radiation, air flow, wind velocity, rainfall, weather conditions and so on (Orihuela, 2000; Mishra *et al*., 2015). Interactions between these factors determined the mechanisms of heat transfer between the animal's body and the surroundings and acted as external regulators of TS (Beg and Totey, 1999).

2.3. SEASONALITY OF THERMAL STRESS

Climatic factors had been found to playcrucial role in the regulation of seasonality of physiological processes in animals. The potential impact of TS on a particular animal species was reflected by the seasonal pattern of reproduction and its association with climatic factors (Hansen, 2009; Kutty and Mathew, 2000). While photoperiod formed the major regulator of circannual rhythm of breeding activity, AbT regulated the circadian and seasonal rhythms through the influence on endocrine and molecular mechanisms of reproductive events (Marai *et al*., 2008). In countries of arid and semi-arid regions, high AbTduring the summer months, and moderate to high AbT together with high humidity contributed by intermittent rainfall of the months adjoining to summer, have been found to be the potential reasons for TS among productive animals such as dairy cattle (Macias-Cruz *et al*., 2016)

Thermal stress resulted in the reduction of feed intake during summer, and together with reduced availability and poorer quality of feed available in tropical countries, exerted a negative influence on adenohypophysis and affected secretion of gonadotrophic hormones leading to weak oestrus and/or anoestrus(Kumari and Pampana, 2015). Fertility of summer and adjoining months (April to September in most tropical countries) was compromised by TS through impairment of oocyte maturation and early embryonic development, while better reproductive performance was observed during seasons of little TS comprised of October to March (Sonmez*et al*., 2005; Dash *et al*., 2016).

Change of seasons caused variations in reproductive performance not only in dairy cattle, but beef cattle as well and was regulated by climatic factors such as AbT, RH, solar radiation and air movements (Beg and Totey, 1999;De-Rensis and Scaramuzzi, 2003). Suppression of the cattle fertility during summer was recorded worldwide and projected considerable economic loss not only because 60 per cent of the cattle were affected but also since the regions affected has been expanding over the years due to the phenomenon of climate change (Al-Katanani *et al*., 1999;Sonmez*et al*., 2005)

In dairy cattle, Krishnan *et al*. (2017) reported that for each unit of THI increase above 70, the conception rate was reduced by 4.6 per centso that overall conception rate of summer was less than 10 per cent. In beef cows Sonmez *et al.* (2005) observed that PP period of dairy cows was longer during autumn than other seasons and was attributed to the persistence of changes in immature follicles caused by summer stress. However, the calving to first insemination interval was not much different between seasons. Ovarian activity decreased during hot summer affecting length of oestrous cycle and degree of expression of oestrus in buffaloes leading to summer anoestrus and was controlled by the combined effects of season, climate, photoperiod, temperature, humidity and nutrition (Beg and Totey, 1999; Kumari and Pampana, 2015).

High AbTformed the major reason for reduced fertility and conception rateof insemination in farm animals during the hot weather in tropical and sub-tropical countries (Das *et al*., 2016). Low percentage of fertilization and reduced viabilityof embryos were found to be the major reasons for lowered conception in cows exposed to warmer climate than cool seasons (El-Tarabany and El-Tarabany, 2015). Conception to artificial insemination (AI) dropped from 40–60per cent in cooler months to less than 20 per cent in summer according to the severity of TS. Moreover, during severe TS, only 10-20 per cent inseminations resulted in normal pregnancies (Das *et al*., 2016). The conception rate of high producing dairy cows in Israel was 45 per cent in winter season compared to 20 per cent in summer (Chebel *et al*., 2004). Krishnan *et al*. (2017) also reported a conception rate reduction of 20–27 per cent during summer for the first service in lactating dairy cows.

Thermal stress has been found to be the major factor responsible for the lowered fertility among lactating dairy cows (Wolfenson *et al.*, 2000), however non-lactating heifers were not seriously affected (Badinga *et al.*, 1985). There occurred amajor reduction in the conception rate of first insemination to reach 7–17 per cent in May to October, from 25–33 per cent in November to April period in Florida (Polsky and Von-Keyserlingk, 2017). In a study in Czek republic, 56.36 per cent conception in cows was recorded during winter season having average AbTof 1.48 °C, while the conception rate dropped to 40.43 per cent at an AbTof 18.96 °C in summer. Assessment of conception variation with AbTrevealed that best conception rate was obtained at temperatures below 5°C. (Kristyna *et al.*, 2017).

Since dairy cattle prosper better in lower AbT, higher conception rates of breeding was recorded in winter season (Bouhroum *et al.*, 2014; Kristyna *et al.*, 2017), and adverse climatic factors of the summer season was found to cause significant alteration of the reproductive rate unless adequate ameliorative measures were incorporated (De-Rensis*et al.*, 2017; Krishnan *et al.*, 2017).

2.4. INFLUENCE OF THERMAL STRESS ON PHYSICAL PARAMETERS

2.4.1. Influence on Dry Matter Intake (DMI)

Over the years milk production of dairy cows has been increasing consistently and was made possible through the planned genetic improvement and better management(Abdalla *et al.*, 2017).Increased milk yield was found to have close association with increase in body weight and feed intake. More feed intake resulted increase of metabolic heat and necessitatedeffective thermo-regulatory mechanisms (Cain *et al.*, 2006) to maintainBT in a thermo-neutral range that ensured physiological homeostasis(Kadzere *et al.*, 2002).

Thermal stress has been found to reduce voluntary feed intake (Allen *et al.*, 2009), as the natural mechanism to cut down the metabolic heat increment as well as to minimize the contribution of heat out of rumen fermentation (Torres-Junior *et al.*, 2008). However, simultaneous with the reduction of feed intake, thermoregulatory system was also activated and increased the maintenance requirement(Guo *et al.*, 2018) to the extend of 7 to 25 per cent, in response to TS of mild to moderate level in dairy cattle as reported by Allen *et al.*(2009). Further, theresultant inadequacy of feed intake to meet the energy demands for maintenance and lactation causedheat-stressed dairy cows to becomein a state of negative energy balance as well (Settivari *et al.*, 2007).

Decrease in DMI to the extent of 23 per cent has been reported in heifers subjected to high AbT(Ronchi *et al.*, 2001). During early PP period, reducedDMIworsened the situation of negative energy balance out of increased milk production, and caused loss of body condition and subsequent reduction of milk yield as well as fertility (De-Rensis*et al.,* 2017; Guo *et al*., 2018). Similar findingwas also reported in ewes by Macias-Cruz *et al*. (2016). However, detrimental effects of reduced DMI was more in lactating cows than non-lactating cows or heifers and was attributed to the increased demand for nutrients to support milk production and associated metabolic processes (West *et al*., 2003; De-Rensis *et al.,* 2017).

Thermal stress caused suppression of fertility in high yielding dairy cows acting many ways, and the major mechanism involved directand indirect effect of altered feed intake. Energy level of feed influenced the productivity and fertility of livestock species in a major way (Krishnan *et al*., 2017). Heat-stressed cows were having reduced blood glucoselevel and at the same time energy expenditure for the maintenance of body condition showed an increase to the extent of 20 per cent at an AbT of 35°C,compared to the thermo neutral condition (Guo *et al*., 2018). Direct influence of negative energy balance involved suppression of follicular waves (Wolfenson *et al*., 1995) and enhanced metabolism of steroid hormones especially progesterone (P4), oestrogen (E2), other metabolic hormones and growth factors caused by increased hepatic blood flow (De-Rensis*et al.*, 2017).

In a study conducted in Algeria on HFcows, failure of AI was found to be related mainly to the nutritional status of lactating cows during cold season as well as TS prone summer months (Bouhroum *et al*. 2014). TS was reported to cause nitrogenous repartition because of the interference of nitrogen metabolism in dairy cow that resulted decreased milk protein content and increase of milk urea concentration (Guo *et al*., 2018).

However, Sonmez *et al.* (2005) reported non-alteration of the DMI at any part of the year because of the TS and the possible reason mentioned was the difference between studies with respect to location, severity of TS and physiological state of cows involved (milk production, BT etc).

The highest AbTprevalent in arid and semi-arid regions of the world has been found to affect reproductive performance of the livestock species directly through TS and indirectly causing poor quantity and quality of feed resources (Krishnan *et al*., 2017). Therefore, ensuring balanced nutrition to livestock forms the fundamental step to achieve satisfactory productivity and reproductive performance in the changing climatic condition.

2.4.2. Influence on Milk Production

The dairy cows with high productivity have been found to be more vulnerable to TS than low producers and was caused by their increased metabolism and greater amount of internal heat generation (Krishnan *et al.*, 2017). The stage of lactation influenced the severity of TS imposed so that decline in milk production due to TS was about 15 per cent in early lactation and reached 35 per cent in mid-lactation (Bernabucci *et al.*, 2010). Prevailing season also influenced the milk yield and chances of getting affected by TS. HF cows during their initial phase of lactation were seriously affected by TS during summer so that the average milk production reached 39.60±5.09 liters as against 42.74±4.98 liters during the spring (Das *et al.*, 2016).

High AbTtogether with high RH during the periods of calving and early lactation was found to exert detrimental effect on milk yield and lactation length (Kristyna *et al.*, 2017; Guo *et al.*, 2018).Such effects were observed not only in pure-bred cattle, but crossbreds, indigenous breeds and dairy goats were also affected and produced greater loss with respect to total yield of milk as well as yield of fat especially in high yielding animals (Hamzaoui *et al.*, 2012; Kumar *et al.*, 2014; Das *et al.*, 2016). Kadzere *et al.* (2002) also reported more adverse effects of TS in high producing cows during early lactation caused by significant reduction of milk yield when rectal temperature (RT)beyond 39.8ºC persisted for more than 16 h compared to late lactation. Berman (2005) reported that cows were highly sensitive to TS due to their increased milk production and for every 10 Kg increase of milk production per day, the threshold for getting affected by TS was found decreased by 5ºC (9ºF).

In lactating cows,AbT as low as 77°F (28.4 ºC) has been found to cause hyper-thermia, even though preferred temperature range for better milk yield in non-pregnant cows was 20.1 to 27.0 °C (Dikmen and Hansen, 2009). However, non-lactating dairy animals, beef cattle and even wild animals were much less likely to be affected by TS at this temperature (Hansen, 2009).Milk yield was found to decreasewhenever the THI value exceeded 72 (Pedersen, 2014). Introduction of THI as a composite measure of TS was made many years back and milk production of dairy cows has achieved tremendous improvement thereafter. Hence, a recent re-evaluation of the TS scenario has suggested the need for lowering the THI threshold to 68 instead of 72 suggested earlier (Allen *et al.*, 2009).

Besides yield of milk, TS also caused alteration of the milk composition such as significant reduction ($P<0.05$) of the milk fat, lactose and protein content (Das *et al.*, 2016).

During TS more amino acids were utilized for maintenance activities including gluconeogenesis and immune response, and resultant decrease of amino acid availability was found to affectmilk protein synthesis as well(Guo *et al*., 2018). Similarly, lowered dry matterintake reduced blood glucose availability and its conversion to lactose during the milk formation (Rhoads *et al*., 2009).Thus overall milk yield was adversely affected,since lactose formed the main osmotic regulator inside the mammary gland (West, 2003). It has been estimated that during hot weather for every unit increase of THI beyond 72, the milk yield decreased at the rate of 0.88 Kg unit (West, 2003).

Hike in productivity of dairy animals has exaggerated their sensitivity to TS, making moreprone to fertility impairments compared to low producers, dry cows and heifers owing to their comparatively lesser metabolic rate (Al-Katanani *et al*., 1999; Al-Katanani *et al*., 2002; Hansen, 2009). Even though the harmful effects of lactation on fertility was acting through different mechanism from that of TS, both appeared to have similar effects on various aspects of reproductive processes such as embryonic development, follicular dynamics and competence of oocytes(Torres-Junior *et al*., 2008). Since reproductive efficiency was having unfavorable genetic correlation with milk yield, higher incidence of late embryonic mortality and early foetal death in high producing cows were attributed to genetic causes (Pryce *et al*., 2004). However, there was significant association of embryonic death with lactation length and milk yield as well (Abdalla *et al*., 2017).

2.4.3. Influence on Body Condition Score

Body condition score (BCS) is a simple tool for assessing the energy status of animals especially dairy cattle. In lactating HF cows Morris *et al.* (2011) described BCS using a scale from 1 to 5 (low to high) with increment of 0.5, wherein animals with mean scoresupto 1.5, 1.5 to 3.0 and more than 3.0 were classified under low, moderate and high BCS groups respectively. Smijisha (2012) put forward a scoring system for crossbred cattle of Kerala, where in instead of observing appearance of the whole body, amount of fat at eight anatomical regions were given special attention for visual assessment of BCS using the 1 to 5 point scale and 0.5 increments.

Nutritional status of the cows had major influence on their fertility so that conception rates of cows with over and under-conditions were found to be lesser, and even if conceived, such animals had lesser chance to maintain pregnancy (Leroy *et al*., 2008). BCS and high somatic cell counts in milk were found to influence postpartum fertility of dairy cows (Morris *et al.*, 2009). BCS of cows recorded at the time of AI was found to have major influence on the pregnancy outcome, with an approximate minimum BCS of 2.5 required for a high probability of conception (Gomez *et al*., 2018). For maximum economy of rearing and as part of scientific management, it is advised to avoid both over conditioning and poor body condition of crossbred cattle at one month prior to calving. The optimum BCS at calving and acceptable maximum loss of BCS upto the second month of calvingsuggested were 3.47 and 0.73 respectively (Smijisha, 2012).

The BCS was found to have positive association with conception and chances for maintenance of pregnancy in cattle (Santos *et al*., 2009). Being an indicator of nutritional adequacy over long time, BCS could be used for making breeding decisions and was useful to predict the potential of the animals to conceive (Gomez *et al*., 2018). Reproductive performance assessed based on services per conception and serum P4 concentration showed significant variation between different BCS groups even though the variation of certain other parameters such as occurrence of first PP oestrus, birth weight of calves and calf mortality during the first three months were not significant (Smijisha, 2012).

In transition cows, BCS was found to be an indicator of metabolic status and helps to predict the reproductive performance. Mobilization of body fat being a key event of transition period to meet the increased energy demand of early lactation, resultant negative energy balance and associated decrease of BCS for several months adversely affected the PP resumption of cyclical activity (Gomez *et al*., 2018). Cows losing BCS from calving to AI were less likely to become pregnant as reported by Santos *et al*. (2009). They also demonstrated the implications of medium and long term changes of BCSon reproductive performance. Long-term energy status of cows assessed based on BCS at AI, was found to reflect pregnancy outcome better than real-time assessment of energy balance. Also, minimum BCS for differentiating cows with optimum reproductive performance from others was found to be around 2.5 (Gomez *et al*., 2018).

Few studies in ewes have showed that body condition status was not affected by elevated AbT of summer months as indicated by relatively constant live weight and BCS across seasons in arid regions of Mexico (Macias-Cruz *et al*., 2013; Delgado *et al*., 2015).In dairy cows Morris *et al.* (2011) observed similar live weights and BCS at the start and end of each season and concluded that these two parameters were not affected by season.

2.4.4. Influence on Body Temperature and Respiration Rate

Respiratory rate (RR) of the animalhas been increasing in response to TS, and was the consequence of the effort for increasing evaporative cooling as the means for dissipation of extra heat load and thus to maintain homeostasis (El-Tarabany *et al.,* 2017). Cardio-respiratory systems of the animals were influenced by weather parameters such as AbT, RH, solar radiation and daytimings (Marai *et al.,* 2007). Increase of THI was found to cause elevation of RR (Sailo *et al.,* 2017) irrespective of the breeds, since cows relied more on respiration as the means for heat dissipation (Rashamol *et al.,* 2018). Hence, RR had been considered as an ideal biomarker of TS (Indu and Pareek, 2015).

The ability of homeotherms to regulate BT relatively constant and function well in spite of marked variations in AbT is contributed by evolutionary adaptation. Accordingly, elevation of RT in response to elevation of surrounding temperature formed an indication of failure to maintain normal BT consequent to TS exposure (Marai *et al.*, 2007; Guo *et al*., 2018). Hence, BT assessed in terms of RT is also considered as a vital biomarker for quantifying TS condition in livestock (Kadzere *et al*., 2002; Rashamol *et al.,* 2018).

Marked elevation of RT and RR in summer than in autumn was reported in many studies (Torres-Junior *et al*., 2008; Morris *et al.,* 2011; Guo *et al*., 2018). In general, environmental variables altered drastically in a day and accordingly physiological responses also showed variation along with time of the day (Guo *et al*., 2018). Physiological parameters such as RR and RT increased from the morning and began to drop from evening until getting stabilized during night hours, corresponding to the diurnal variations of the weather parameters (Rashamol *et al.,* 2018).

Rhoads *et al*., (2009) observed that cows under TS were exposed to persistent elevation of AbTfor the entire day so that RR and RTshowed peak differences during the afternoon that ranged from 46 to 82 breaths/min and 38.7 to 40.2°C respectively. Bolocan (2009) observed a 3 to 3.5 times increase of the RR at 4 pm compared to the recording at 7 am, in response to increasing AbT, and correspondingly, RT raised 1.5 to 2 degrees above the normal value indicating high level of stress.

In response to TS, various compensatory mechanisms were found to be initiated to regulate BT including enhancement of cardio-respiratory functions (Marai *et al*., 2007). Whenever, increased RR and a peripheral blood flow failed to dissipate the excessive heat load, there occurred elevation of RT as well (Cain *et al*., 2006). RT was found to be 0.68 ºC higher in the TS group than control (Torres-Junior *et al*., 2008). Persistent elevation of RT was found to initiatevarious manifestations of TS such as reduction in feed intake, increased intake of water and hormonal imbalances. Impairment of reproductive processes also followed such as altered oestrus manifestation, ovulatory disturbances, corpus luteum (CL) dysfunction and failure of implantation (Marai *et al*., 2008; Rhoads *et al*., 2009; Macias-Cruz *et al*., 2016).

The elevation of RT (>39.4°C)around the time of AI during the warm season wasfound to be associated with lowered conception rate in cows (De-Souza *et al*., 2016). For every unit increase of RT around the time of AI, conception rate was found to suffer major reductionattributable to the inability of the cows to maintain normal BT at highAbT(Allen *et al.,* 2009). Elevation of RT around 72 h after insemination from 38.5 to 40°C was also found to reduce the pregnancy rate in dairy cows up to 50 per cent(Krishnan *et al*., 2017).

Both RT and conception rates were affected by seasons manifesting higher RT and lower CR during hottest months of the year than the autumn-winter months. Increase of RT during summer months due to the elevation of AbThas been found to compromise the reproductive efficiency of dairy cows, especially of *Bos taurus* breeds (De-Souza *et al*., 2016), while *B. indicus*cattle are more resistant. Such adaptation of *B. indicus*breeds to elevated AbTand RH was found to be mostly due to their superior ability toregulate BT (Gaughan *et al*. 1999) together with intrinsic cellular resistance against varying level of TS (Torres-Junior *et al*., 2008)].

2.5. INFLUENCE OF THERMAL STRESS ON REPRODUCTIVE PERFORMANCE

Thermal stress has been found to cause disruption of various physiological processes, which include not only those changes with immediate consequences emerging during the period of high temperature such as reduced milk yield, but long lasting effects as wellsuch as impairment of fertility (De-Rensis*et al*., 2015). TS had its harmful effects on almost all aspects of reproductive function in mammals including spermatogenesis, development and maturation of the oocyte, fertilizable life of gametes, embryonic development, endometrial function, steroidogenesis, uterine blood flow, foetal and placental growth and initiation and maintenance of lactation (Wolfenson *et al.,* 2000; De-Rensis and Scaramuzzi, 2003). Impairment of these processes occurred either due to the direct influence of hyperthermiaaffecting normal occurrence of the reproductive events, or indirectly causing disruption of the homeo-kinetic system in regulating reproductive processes(Hansen, 2009).

Thermal stress caused disruption of endocrine regulation of reproduction including hypothalamo–hypophyseal–ovarian axis. Hyper-prolactinaemia consequent to TS acting at the hypophyseal level caused inhibition of the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH)andaffected various reproductive functions leading to lowered reproductive efficiency (Kumari and Pampana, 2015). Similarly, follicular codominance and resultant occurrence of shortened oestrous cycle length was reported to affect the functional competence of oocytes (El-Tarabany and El-Tarabany, 2015). In lactating dairy cows, Das *et al.* (2016) reported that conception rate of AI reduced from 40 – 60 per cent in cooler months to 10-20 per cent consequent to and depending upon the severity of TS. Moreover, during the months of severe TS, only 10-20 per cent of inseminations resulted in normal pregnancies (Das *et al.*, 2016).

Harmful effects of TS on oocyte development during early folliculogenesis has been found to persist for about 16 weeks after the initial exposure so that disruption of fertilityhappened during summer was restored very slowly only in autumn period (Das *et al.*, 2016). However, the possibility of enhancing fertility in the autumn by aspiration and removal of those follicles damaged by TS in the previous summer was realized by De-Rensis*et al.*(2017). Harmful effects of TS on reproductive processes were more pronounced in *Bos taurus* cattle (El-Tarabany and El-Tarabany, 2015), while more thermo-tolerant *Bos indicus*cattle was less affected by immediate effects of TS, and needed comparatively longer exposure for developing impairment of fertility (Torres-Junior *et al.*, 2008).

Some previous studies had reported peri-implantation stage as the most vulnerable period causing considerable decrease of conception rate, which was manifested through high incidence of implantation failure since TS caused dramatic morphologic changes on embryo and the endometrial stroma (El-Tarabany and El-Tarabany, 2015). However, in lactating dairy cows, there was a negative influence of TS on the conception rate both before and after the day of service and the period started from 42 days before and until Day 31 post-service(Schuller *et al*., 2014). Krishnan *et al*. (2017) also reported deleterions effect of TS on conception starting from 42 days before and upto 40 days after insemination.An increase of 0.5°C in the uterine temperature during hot days resulted considerable reduction in fertilization rate and most damages to the conceptus due to exposure of cows to severe TS occurred between Day 0 and Day 7 of oestrus(Alejandro *et al*., 2014). Anyhow, the extended period of fertility reduction consequent to TS has become the most serious concern of dairy farmers worldwide in the context of global warming.

2.5.1. Influence on Postpartum Fertility

During early PPperiod dairy cows has been under extreme stress of metabolic adjustments. Exposure to TS at this stage delayed the days open due to decrease in the oestrus shown, reduced conception to AI and lesser establishment of pregnancy(Nardone *et al.,* 2010). This was attributed to the reduced DMI and associated negative energy balance, reducing the fertility and thus prolonging the PP period (Jonsson *et al*., 1997). Reduced fertility of summer attributed to TS was caused by various factors such as impairment of cellular function, altered metabolism in various tissues, compromised ovarian follicular dynamics and failure of primary follicles to dominate (Torres-Junior *et al*., 2008).

Postpartum fertility varied between different types of animals, high producers being more affected. Pure HF cows has been found more affected with a significantly long inter-calving interval and days open upon exposure to high THI than low THI. Whereas BS and their crossbreds with HF had little difference in the inter-calving interval at different THI levels(El-Tarabany and El-Tarabany, 2015). Sonmez *et al.* (2005) found no difference in the PP fertility of dairy cows between seasons. Where as in beef cows, the interval from calving to first service was longerduring autumn than other seasons and the difference was statistically non-significant. However, in buffaloes occurrence of more silent oestrusrather than anoestrus during summer contributed for longer service period(Krishnan *et al*., 2017).

Parity was found to affect milk production, metabolic status and P4 profiles in PP cows but there was no influence on prolongation of PP anoestrus (Morales *et al*., 2018). TS was found to affect the competence of oocytes for fertilization and subsequent development and ended up with lowered fertility(Krishnan *et al*., 2017). Culturing of granulosa and thecal cells exposed to TS 20 - 26 days before harvest, evidenced lowered steroid production capacity (Roth *et al*., 2001b). In lactating dairy cows removal of those follicles started growth during summer stress, was found to hasten the resumption of fertility during autumn(Hansen, 2009).

Among cows exposed to chronic stress of lameness from the early PP period, 29 and 21 per cent were affected by lack of ovarian activity and failure of oestrus expression respectively while 50 per cent were unaffected with respect to various reproductive parameters (Morris *et al*., 2011). Also, low first AI pregnancy rate and more number of AI per conception were other manifestations of cows affected with lameness continuously for several weeks(Hernandez *et al.,* 2001; Morris *et al*., 2011). Even though there was no immediate effect of TS on reproductive performance of Gir cowsduring early PP period, there occurred delayed effect on follicular growth,oocyte competence and hormone concentration manifested by longperiods of non-cyclic activityand/or short cycle (Torres-Junior *et al*., 2008). Alterations of follicle formation, impairment of oogenesisand delayed ovulation also occurred and resulted longer calving interval, low birth weight of calves and lowered milk production (Krishnan *et al*., 2017).

Summer season harvested oocytes were having reduced ability to fertilize and develop into the blastocyst stage after *in vitro* fertilization, than those harvested in winter (Rocha *et al.,* 1998; Al Katanani *et al.,* 2002). Similarly proportion of abnormal and developmentally retarded embryos were more in HF heifers exposed to TS between the onset of oestrus and AI when compared to heifers maintained at thermo-neutrality (Torres-Junior *et al*., 2008). After TS exposure, appearance of competent oocytes necessitated two or three oestrous cycles after the end of stress exposure. This indicated the delayed deleterious effect of TS on ovarian function so that follicles and oocytes could be damagedduring early stages of folliculogenesis, and restoration of normal ovarian function is delayed at least for the duration of two or three cycles (Torres-Junior *et al.,* 2008).

2.5.2. Influence on Oestrus Characteristics

Oestrus in mammals is the period of sexual receptivity characterized by the occurrence of behavioural signs in the female that facilitate mating close to the time of ovulation. In both male and female animals, extreme AbT has been found to delaythe onset of puberty and in PP females resumption of cyclicity was delayed (Krishnan *et al*., 2017). Moderate elevation of AbThas been reported to affect the behavioural and physical manifestations of the oestrus so that as many as 80 per cent of oestrus goes undetected (Roelofs *et al*., 2010; Das *et al*., 2016).

Exposure to high AbThas been reported to shorten the duration and intensity of oestrus signs in cattle (Lopez-Gatius *et al*., 2005b, Sakatani*et al.,* 2012a). Along with marked seasonal variations of oestrus signs exhibited, many ovulations occurred without any behavioural signs during summer months (Lopez-Gatius *et al*., 2008; Roelofs *et al.,* 2010). Elevation of THI beyond 63 was found to cause proportionate reduction of oestrus behaviour including secondary signs in cows during summer months and incidence of anoestrus and silent ovulation increased considerably beyond a THI of 78 (Nmez *et al*., 2005; Schuller *et al.*, 2017).

Thermal stress inhibited the growth of pre-ovulatory follicles and resulted in decreased secretion of oestradiol starting from pro-oestrus and resulted poor manifestation of oestrus signs (Wilson *et al*., 1998; Sonmez*et al*., 2005; Hansen, 2015). Steroid production capacity of theca and granulosa cells were inhibited by TS leading to diminished oestradiol concentration in the circulation so that oestrus signs were weakened making detection difficult/failed especially during summer (Sonmez*et al*., 2005; De-Rensis *et al*.,2015). TS also caused reduced secretion of LH, affected the length and intensity of oestrus behaviour and also delayed the ovulation (Pedersen, 2014;Krishnan *et al*., 2017).

Mounting activity of oestrus was considerably decreased during summer than other months and the activity was mostly shifted towards night hours (Sakatani*et al.*, 2012a; Alejandro *et al*., 2014; Krishnan *et al*., 2017). Secretory activity of endometrium was also reduced by TS affecting expulsion of cervical mucous during oestrus (Yaniz *et al*., 2004), so that detection of vaginal mucous flow as a useful sign for heat detection was also reduced (Roelofs *et al.*, 2010).

Thermal stress affected physical activities of the animal including walking so that detection of oestrus was reduced even with the use of oestrus detection devices (Lopez-Gatius *et al*., 2005b; Roelofs *et al.*, 2010; Sakatani*et al*., 2012a). When hot days (33-38ºC) alternated with tolerable temperatures during night hours (18-21ºC), normal expression of oestrus was noticed only in 16.7 per cent of the animals, while 33.3 per cent experienced weak oestrus and 50 per cent did not exhibit oestrus behaviour at all (Bolocan, 2009). In conditions of moderate TS, with peaks of daily temperature ranging from 28 to 34ºC, 25 per cent of the heifers had normal expression of oestrus, while 41.7 per cent had weak symptoms and in 33.3 per cent, oestrus became silent (Bolocan, 2009; Krishnan *et al*., 2017).

TS affected the development of follicle during the antral and pre-antral stages and also increased the rate of apoptosis (Krishnan *et al*., 2017). First PP ovulation was reported to occur often without accompanying behavioural signs. Also there was reduced intensity of oestrus in high milk yielding animals (Roelofs *et al.*, 2010). Both these conditions were believed to occur due to the inadequacy of P4 priming, caused by the lack of preceding ovulation in pre-pubertal/post-partum stages and rapid clearance of P4 by the enhanced steroid metabolism in case of high yielding cows (Roelofs *et al.*, 2010).

In the case of ewes, Macias-Cruzs *et al*. (2016) reported that oestrus signs, ovulation rate and average length of oestrous cycle was more or less similar in summer and autumn without obvious seasonal effects. However, intensity of oestrus signs were significantly affected by the seasons in dairy and beef cows, with decreased expression during summer as against increased expression during spring (Sonmez *et al*., 2005; Sakatani*et al*., 2012a). Correspondingly, fertility also varied between seasons mainly due to failure of detection, altered endocrine profile and increased secretion of $PGF_2\alpha$ (prostaglandin F_2 alpha) from the endometrium (Das *et al*., 2016).

Other than season and climate, type of housing and management and various animal factors such as milk yield, lameness, social interactions and vicinity of males were found to influence the expression of oestrus behaviour (Roelofs *et al.,* 2010; El-Tarabany and El-Bayoumi, 2015). There was significant difference in the duration of standing to be mounted depending upon the type and nature of flooring of the exercise yards (Rodtian *et al.,* 1996). Therefore, additional strategies were needed to improve heat detection in animals confined to barns / yards and to counteract the adverse effect of TS affecting detection of oestrus across different seasons and varied management situations (Sonmez *et al*., 2005).

Detection of oestrus with reduced intensity and duration of manifestation has been mentioned to be a major problem despite lot of advancements in reproductive management strategies andavailability of oestrus detection aids for cattle(Krishnan *et al*., 2017). In order to achieve better oestrus detection rates in such animals, observation of various secondary changes were necessitated either visually or using adequate detection aids (Van Eerdenburg *et al.,* 1996; Walker *et al*., 2008; Roelofs *et al.,* 2010). Maximum display of oestrus behaviour was reported to occur in the morning and midnight, when the AbT was low (Nmez *et al*., 2005; Kumari and Pampana, 2015), and more attention at these timings was necessitated to achieve better detection rates.

For the enhancement of oestrus detection efficiency, different methods of oestrus intensity assessment were utilized. Van-Eerdenburg *et al.* (1996) suggested an oestrus intensity scoring system for dairy cattle reared freely under semi intensive management. In this system many factors associated with oestrus manifestations were taken into consideration even though major emphasis was given for standing to be mounted. Azeez (2014) used a different scoring system for oestrus intensity assessment in dairy cattle managed under intensive system wherein the animals were confined in barns all the time so that there was lack of opportunity for the expression of major behavioural signs. Meenuja (2017) and Shakir (2018) also utilized the same scoring approach for oestrus detection under intensive management.

2.5.3. Influence on Growth of Ovarian Follicles

On functionally normal and active ovaries of cattle, growth of small follicles occurred simultaneously in batches of two or three and advanced one after the other during each oestrous cycledesignated by the term follicular wave (Ginther *et al.,* 1989). Each follicular wave was starting with a cohort of few follicles of >4 mm in diameter and often one folliclewas selected to become dominant and others disappeared through atresia (Ginther *et al.,* 1996). However, consequent to the failure of dominant follicle that developed during the luteal phase to ovulate, the successive one with some developmental advantage at the onset of luteolysisbecomesdominant and ovulate during the subsequent follicular phase (Goeseels and Kastelic, 2003).

Among the cohort of small follicles recruited to grow, any follicle that reached a critical stage of growth at first than the immediate successor was emerging out to become the dominant follicle.Thus, the dominance was not decided by the advantage of size alone but any follicle that attained 5 to 7 mm regardless of the size ranking might become dominant unless it was actively suppressed by a predecessor (Ginther, 2016). In cows with two follicular waves, oestrous cycle was shorter and ovulatory follicle was larger, older and produced more oestradiol than ovulatory follicle of cows with three waves (Ginther *et al*., 1989; Goeseels and Kastelic, 2003).

Size of the dominant follicle varied depending upon so many internal and external factors such as breed, milk production, dry matter intake and climate (Roth *et al*., 2000; De-Rensis *et al.,* 2017). Consequent to reduced dry matter intake during summer, lowered plasma concentration of glucose, insulin and insulin like growth factor-1 were found to interfere with the development of follicles and resulted smaller size (De-Rensis*et al*., 2002). Sartorelli *et al*. (2005) compared different stages of ovarian follicles between *Bos taurus* and *Bos indicus* and reported the size variations. While medium sized follicle of *Bos taurus* was 8.5 mm, corresponding size in *Bos indicus* was 6 mm forming only 70 per cent of the size in *Bos taurus* (Ginther *et al.,* 1996;Sartorelli *et al*., 2005; Gaur and Purohit, 2007; Ginther, 2016). Hence, a proportionate reductionof the size was expected for other stages of follicles as well in *Bos indicus* cattle.

Follicular dynamics has been altered during TS and there appeared to have an inverse relationship between TS and follicular size demonstrated by a 0.1 mm decline in follicular size for every unit of THI increase on the day of oestrus (Schuller *et al*. 2017). TS was found to be associated with an increase of plasma FSH and decrease of inhibin, alteringfollicular dynamics and thus affecting fertility of cows during the summer and autumn(Roth *et al*., 2000; Hansen, 2009). Such an elevation of FSH during TS was probably due to weakened inhibition of negative feedback caused by reduced secretion of inhibin from smaller follicles (Khodaei-Motlagh *et al.*, 2011; Das *et al*., 2016).

In lactating cows exposed toTS, thedominant follicle of first wave was smaller and contained less fluid than that of controls (Badinga *et al*., 1993) and was found to be caused by the early occurrence of second wave dominant follicle(Wolfenson *et al*., 1995). Such follicles were found to be persisting longer and resulted reduced fertility since duration of persistence of the pre-ovulatory follicle had a negative correlation with fertility (Goeseels and Kastelic, 2003). Also size of the follicle from which the occytes originated was shown to have a positive association with fertilizing capacity and developmental competence of the embryos (Trout *et al*., 1998; Austin*et al*., 1999;Goeseels and Kastelic, 2003).

Thermal stress during the phase of follicular growth interfered with the recruitment as well as subsequent growth and development of dominant follicles to attain the capability for ovulation (Roth *et al.*, 2000; Krishnan *et al*., 2017). Attainment of fertilization capacity of the occytes was also disturbed (Hansen, 2009).Ovulation of small follicles was found to result smaller CL(11.5 ± 0.2 mm) that secreted low amount of P4and resulted lowered conception rates when compared to follicles of larger size (14.5 ± 0.2 mm) as reported by Vasconcelos *et al*. (2001). Major mechanism for lowered conception in such cases was found to be through the reduced production of interferon-τ (IFN-τ) by the conceptus so that maternal recognition of pregnancy was interfered (Goeseels and Kastelic, 2003)

Besides thermal influence, any form of stress such as diseases, chronic lameness and other harsh environments were found to affectthe size and steroidogenic capacity of follicles and quality of the oocyte inside (Roth *et al*., 2000; De-Rensis*et al.*, 2017). Consequently oestrus expression, LH surge, ovulation and embryonic development were also disturbed and resulted inlowered fertility (Morris *et al.*, 2011). El-Tarabany and El-Bayoumi (2015) reported that stressful conditions including lameness caused failure to express oestrus or failed to ovulate in 21 per cent of cows in spite of having an apparently normal follicle.

Further,incapability to produce a functionally normal follicle in response to exogenous hormonal stimulation was also observed in 29 per cent of lame animals and attributed to the influence of stress (El-Tarabany and El-Bayoumi, 2015)

In addition to the size alterations, TS in dairy cows also affected the number and dominance of the follicles recruited. While the presence of medium-sized subordinate follicles were increased, functional capabilities of the dominant follicle was adversely affected (Roth *et al.*, 2000; Roth *et al.*, 2001a; Sartori *et al.*, 2002). There were reports of increasing, decreasing and no difference in the number of small (2 to 5 mm) follicles in response to TS (Badinga *et al.*, 1993; Wolfenson *et al.*, 1995; Trout *et al.*, 1998; Wilson *et al.*, 1998; and Wolfenson *et al.*, 2000). Induction of early emergence of the second wave follicle because of TS was causing inadequacy of follicluar dominance and increased the risk of conception failure due to delayed ovulation (Wolfenson *et al.*, 1995; Austin*et al.*, 1999)

Prolonged dominance by small sized follicles was causing disruption of the normal oocyte maturation that leadto ovulation of the sub fertile oocyte, functionally incompetent corpora lutea and regression of many premature follicle resulting substantial reduction in ovulation percentage (Ronchi *et al.*, 2001; Krishnan *et al.*, 2017). De-Rensis *et al.* (2015) observed that in cows affected by ovulatory disturbances, CL did not develop or there occurred persistence of pre-ovulatory follicle with 8–15 mm diameter and in many cows, expanded further (18–32 mm diameter) to resemble ovarian cysts (Lopez-Gatius *et al.*, 2005a; De-Rensis*et al.*, 2015). Ronchi *et al.* (2001) reported that exposure to high AbT was leading to the development of ovarian cysts due to impairment of follicular dynamics. Wolfenson *et al.*(1995) also reported more number of large follicles not only from the initial follicular wave but because of advance emergence of the second follicular wave as well and there was a corresponding decrease in medium-sized follicles.

Long term influence of TS was found to cause ovarian hypo-function leading to reduced growth of follicles and many of the follicles undergoing atresia among primary to tertiary follicles (Kumari and Pampana, 2015). In thermo-tolerant breeds of cattle, TS exposure did not cause any immediate effecton reproductive performance, however there were long term consequences affecting growth of follicles and competence of oocytes leading to impairment of fertility (Torres-Junior *et al.*, 2008).

2.5.4. Influence on Oestrogen Level

During the oestrous cycle, E2 produced mainly from the granulosa cells of the follicle has been producing various changes in ovaries like luteolysis and suppression of the growth of subordinate follicles (Schuller *et al*., 2017). In high producing cows consumption of more feed resulted dramatic increase in the liver perfusion and faster metabolism of ovarian steroids (Wiltbank *et al*., 2006) that lead to a slow increase and inadequate peak of oestradiol, and poor quality oocytes which failed to undergo embryonic development upon fertilization (Sakatani *et al*. 2012a; Abdalla *et al.*, 2017).

Thermal stress hasbeen found to affect the length of oestrous cycle in dairy cows and heifers (Torres-Junior *et al*., 2008). Even though there were differences among reports regarding the effects of TS on steroidogenesis (Wilson *et al.,* 1998; Gendelman*et al.,* 2010), most of the recent studies reported a reduction in the level of E2 consequent to TS (Ozawa *et al*., 2005; Torres-Junior *et al*., 2008). This was found to be due to the low production of androstenedione and reduced aromatase activity (Roth *et al.,* 2000; Wolfenson *et al.,* 2000). Schuller *et al*. (2017) mentioned that failure of adequate oestradiol secretion consequent toTS affected the normal mechanism leading to luteolysis. As the result, development of dominant follicle of the cycle was inadequate and also caused smaller size of the ovulatory follicle in the subsequent cycles as well.

Thermal stress was found to delay the recruitment of ovulatory follicles in goats and led to lowered plasma oestradiol concentration, even though the concentrations of FSH and P4 remained the same (Ozawa *et al*., 2005). TS was found to damage even the developed follicles, making them non-viable because of the elevation of BT above 40°C (Roth *et al.*, 2000). Similarly,female goats exposed to AbTof 36.8°C with RH of 70 per cent for 48 h suppressed follicular growth, oestradiol synthesis and ovulation accompanied by decreased level of LH (Ozawa *et al*., 2005; Das *et al*., 2016). Granulosa and thecal cells collected from cows exposed to TS during the previous 20–26 days, showed less secretory activity for oestradiolin culture (Roth *et al*., 2001b).

Oestradiol secretion in buffaloes has been reported to have two distinct patterns depending upon the management situation, such as a low level in the peripheral circulation attributed to summer anoestrus caused by environmental stress,and a higher level during winter (Kumari and Pampana, 2015). In TS animals, reduced plasma concentrations of oestradiol-17-β and inhibin and increased plasma concentrations of FSH was found to cause loss of follicle dominance (Roth *et al*., 2000; Torres-Junior *et al*., 2008).Lowered estradiol secretion by the follicle and consequent reduction of uterine blood flow during TS has been found associated with reduced follicle size and was demonstrated by a significant increase of uterine blood flow in cyclic cows within 30 min of estradiol injection(Schuller *et al*., 2017).

Wolfenson *et al*. (1997) reported highest mean level of oestradiol in autumn, lowest in summer and intermediate in winter. Short term TS during winter months caused 46 per cent reduction of the oestradiol production compared to autumn levels, even though between TS and control groups there was no significant difference in oestradiol productionduring winter (Wolfenson *et al*., 1997). Seasonal differences in steroid production capacity of follicles were affected by factors such as feed intake and gonadotropin secretion which affected the secretion of steroids by the follicles (Wolfenson *et al*., 1997; Roth *et al*., 2001b).

Mechanism of TS effects on follicular function involved changes at the level of follicle and also on the secretion of pituitary hormones. Bovine follicular cells in culture produced lower levels of steroid at elevated temperature (Wolfenson *et al*., 1997; Bridges *et al*., 2005). In addition, follicular responsiveness to LH, as measured by oestradiol release after injection of gonadotropin releasing hormone (GnRH) was reduced by TS in goats. Also in rats TS was found to reducethe gonadotropin receptors and aromatase activity of granulosa cells and concentration of oestradiol in the follicular fluid (Shimizu *et al*., 2005).

Animals exposed to TS had a significantly lower level of E2 during Days 4-8 of first follicular wavewhen the largest follicle exerted its dominance. This reduction in E2 concentration may serve as an additional indication of altered follicular function and dominance under conditions of TS (Wolfenson *et al*., 1995)

2.5.5. Influence on Breeding and Fertility

Extreme elevation of AbTdelayed the puberty in both male and female animals (Krishnan *et al*., 2017). Besides decrease in milk production, impairments of reproductive processes caused by TS were found to cause considerable economic loss (De-Rensis*et al.,* 2015).The effects of TS on dairy cow fertility have been widely established to be causative of prolonged days open, decline in conception rate, high proportion of anoestrus, more incidence of ovulatory disturbances, persistent follicles and ovarian cysts (Wolfenson *et al*., 2000; Kadzere *et al*., 2002; De-Rensis *et al*., 2015). In a large scale retrospective study, Lopez-Gatius (2003) observed that average of first insemination pregnancy rates decreased from 44 per cent of winter to 27 per cent in summer, while anoestrus proportion increased from 1.2 per cent to 12.9 per cent during the same periods.

Pregnancy rate of AI in pure HF cows dropped significantly from 28.5 per cent at low THI to14.8 per cent at high THI and simultaneously foetal loss rate showed significant increase from 17.1 to 24.9 per cent. Most other reproductive performance parameters were also showing similar variation between THI periods in this breed (El-Tarabany and El-Tarabany, 2015). In dairy cows elevation of THI above 72 was found to reduce the conception rate of AI and pregnancy rate, while in the case of buffaloes a significant reduction in reproductive performancewas observed only beyond THI 75. TS compromisedoocyte quality from as early as 105 days before ovulation (Torres-Junior *et al*., 2008) and extended beyond the ovulatory period causing incompetency of embryos produced (Torres-Junior *et al*., 2008; Krishnan *et al*., 2017).

In lactating dairy cows, conception per insemination has been found to be as low as 10-20 per cent during summer, and such a reduction was attributed to the damage caused on oocyte and early embryo (Hansen, 2015). Lopez-Gatius (2003) reported that dairy cows inseminated during warm months of the year achieved a pregnancy rate of 22.1 versus 43.1 per cent during cool season. However, the magnitude of the fertility decline during summer was much less for heifers, dry cows and low yielders, when compared to cows with high milk yield (Al-Katanani *et al*., 1999; Hansen, 2009)

Thermal stress around the day of breeding had its immediate effect by reducing conception rate and delayed effect was reported upto 40 days after AI (Jordan, 2003; Ispierto *et al*., 2007; Morton *et al*., 2007). Delayed influence of summer TS on fertility was evidenced by the lowered fertility during fall compared to winter, even after AbTdropped in the fall periodand cows were no longer exposed to TS (Wolfenson *et al*., 1997).

2.5.6. Influence on Progesterone Level

Progesterone produced by the CL during the oestrous cycle and pregnancy is essential for the establishment and maintenance of pregnancy (Lucy, 2001; Gomez *et al*., 2018) until completion of the gestation period. Maternal recognition of pregnancy leads to prevention of luteolysis so that growth and secretory function of CL was maintained and P4 level continued to increase. P4 level of cows that maintain pregnancy was 2.4 to 4.2 ng/mL, while in those suffered embryonic loss was 1.93 ng/mL(Starbuck *et al*., 2004; Siregar *et al*., 2017).

TS has been reported to reduce the length of the oestrous cycle, together with reduction of P4 secretion in cows and heifers (Wilson *et al.,* 1998) and was found to be associated with reduced secretory activity of theca and granulosa cells in both *in vitro* and *in vivo* studies (Wolfenson *et al.,* 2000). Low P4 secretion affected embryonic development through impairment of endometrial functions including increased secretion of endometrial $PGF_{2\alpha}$ and caused luteolysis and pregnancy loss (Krishnan *et al*., 2017).

Ronchi *et al.* (2001) observed that heifers under restricted feeding had smaller CL compared to *ad libitum* fed heifers, even though P4 concentrations did not differ. In addition to impairment of P4 secretion by the luteal cells, TS also caused reduced ovulation rate and affected CL formation in causing reduced fertility during the warm climate (Lopez-Gatius *et al.,* 2005a; Torres-Junior *et al.,* 2008).Macias-Cruz*et al.* (2016) reported significant reduction in the P4 concentration especially between days 8 and 14 of the oestrous cycle, during summer compared to autumn season, and was due to decreased functionality of CL, even though the oestrus and ovulatory activities were unaffected.Higher levels of serum P4 on days 6, 13 and 20 post-AI was reported in conceived animals by Gomez *et al*. (2018).

Elevated AbT has been found to have adverse effects on embryonic survival *in utero*and was attributed to, rather than direct effect on the embryo, various changes in maternal physiology - the major onebeing reduction in the circulating P4 concentrations (Wolfenson *et al*., 2000; Hansen, 2009). Various earlier studies on the effects of TS on the plasma P4 levels have generated contradictory findings in dairy cows and ewes (Macias-Cruz *et al*., 2016; De-Rensis*et al.,* 2017). P4 levels were reported to have increased (Trout *et al*., 1998; Wilson *et al*., 1998; Marai *et al*., 2007; Sejian *et al*., 2013a), decreased (Wolfenson *et al.,* 2000; Alnimer *et al*., 2002; De-Rensis*et al.,* 2008) or unchanged (Roth *et al.,* 2001; Guzeloglu *et al*., 2001b; Ali and Hayder, 2008) between animals exposed and not exposed to thermal stress.

Thermal stress has been found to reduce the plasma P4 concentrations in dairy cows and heifers (Ronchi *et al*., 2001; Torres-Junior *et al*., 2008) and was attributed to increased hepatic break down of steroids (Gomez *et al*., 2018), premature luteolysis and cessation of steroid secretion initiated by increased secretion of $PGF_{2\alpha}$from the endometrium (Lopez-Gatius *et al*., 2005a; De-Rensis*et al.,* 2017). Macias-Cruz *et al*. (2016) also reported decreased blood P4levels in cows exposed to TS during summer compared to those in a thermo-neutral environment during autumn.

In this regard, Wolfenson *et al*. (2000) reported that while acute TS lowered the P4production, chronic TSresulted in elevation of the levels. TShas been found to exert delayed deleterious effect on ovarian follicular growth, hormone concentrations, and oocyte competence in Gir cows. Longer periods of non-cyclic activity as well as shorter oestrous cycles were also reported in TS exposed cows (Torres-Junior*et al*., 2008).Another possible explanation for the reduction in P4 concentration and lowered fertility in cows exposed to hot environment was the reduced availability of plasma cholesterol owing to the impairment of lipid metabolism in cows (Ronchi *et al*., 2001)

During summer, TS caused decreased secretion of GnRH and gonadotropins and inhibition of ovarian activitythrough the influence on activity of the hypothalamo hypophyseal gonadal axis (De-Rensis*et al.*, 2017). TS also affected basal level of P4 secretion by both large and small lutein cells causing altered seasonal pattern. According to Schuller *et al*. (2017), exposure to TS delayed functional luteolysis in cows compared to those under thermo-neutral conditions so that P4 concentration was higher after Day 16 of the oestrous cycle (3.5 vs < 1 ng/mL) in TS cows. They observed that increasing THI above 74 on the day of oestrus reduced the chances of getting a serum P4 concentration of < 1 ng/ml after Day 16 of the cycle.

In lactating dairy cows, poor pregnancy rate as a result of low P4concentration was found to be benefitted throughP4 supplementation after AI, by improved pregnancy rates (Starbuck *et al*., 2004; Gomez *et al*., 2018). Ronchi *et al*. (2001) documented a reduction in serum P4 concentrations in TS affected cows during the luteal phase and attributed the same to lowered number of luteal cells under high AbT. Poor quality oocytes consequent to abnormal steroid profile during TSfailedto undergo normal embryonic development andwas further complicated by altered uterine environment also contributing to the failure of embryonic or fetal growth(Abdalla *et al.*, 2017).

In buffaloes, reproductive performanceis considerably reduced during summer and is found to be contributed by the reduced activity of luteal cells manifested through reduced P4 secretion of summer months compared to normal breeding season as well as winter (Kumari and Pampana, 2015).Plasma P4 concentrationconsequent to TS varied depending upon the nature of stress factor. In vitro studies have shownthat luteal cells collected during summer produced low levels of P4compared to those collected in winter. Low plasma level of P4 caused various effects including impairments of steroidogenesis in the dominant follicle as well as in the CL, maturational defects of oocytes and changes in endometrial morphology interfering embryonic development (Goeseels and Kastelic, 2003).

2.5.7. Influence on Conception

It is well documented that fertility of dairy cattle has decreased continuously in the past 30 years together with the increase of milk production (Butler, 2000; Lopez-Gatius, 2003), and TS was found to be one of the major contributory factors. TS was found to reduce the fertilization rates in cattle even upto the extent of 80 per cent (Saacke *et al*., 2000). The influence of TS on mammalian reproduction can be understood by studying the seasonal trends in fertility performance (Hansen, 2009). In countries having predominant summer, conception rate of insemination in dairy cows was reduced from 43.1 per cent during cool season to 22.1 per cent during warm months of the year (Lopez-Gatius, 2003; Hansen, 2009).

Thermal stress has been found to interfere with the physiological mechanisms following fertilization that leads to conception and maintenance of pregnancy (Wolfenson *et al.,* 2000; De-Rensis and Scaramuzzi, 2003). Elevation of AbTduring the breeding period was found to have a negativeassociation with conception rate (Schuller *et al.,* 2014; De-Souza *et al*., 2016).Impairment of conception caused by TS could persist as long as the small antral follicles of the stress period developed into large dominant follicles and ovulate which might take 40-50 days (Sonmez *et al*., 2005). TS induced impairments of steroidogenesis in ovarian follicles and CL lead to reduced P4 secretion, endometrial dysfunctions, elevated endometrial $PGF_{2\alpha}$and reduced uterine blood flow which caused increased occurrence of embryonic mortality (Abdalla *et al.,* 2017; De Rensis *et al.,* 2015).

Harmful effects of TS on conceptus were occurring mostly during the initial stages of embryonic development (Demetrio *et al.,* 2007; De-Rensis*et al.,* 2017). In lactating dairy cows, proportion of embryos developing to blastocyst was considerably reduced by the TS exposure during the metoestrus period (Das *et al*., 2016). Even though oocytes were less affected by prolonged period of TS, embryos were found much vulnerable during the peri-implantation stage being the period critical for implantation (Sakatani *et al.,* 2012a; Ispierto *et al.,* 2007). TS also affected the embryonic production of IFN-τ, which was found highly essential for the prevention of $PGF_{2\alpha}$ secretion from the endometrium and maintenance of CL for successful pregnancy (Spencer *et al.,* 2007; Pedersen, 2014).

Sensitivity of cattle embryos to TS was maximum during the first two weeks after breeding (Pedersen, 2014; Ahmed *et al.,* 2015) and elevation of AbT did not exert much harmful effects on the embryos after the implantation (De Rensis *et al.*, 2015). However, it was reported that conception failurecould occur over a period of 42 days before and 40 days after the date of insemination (Sonmez *et al*., 2005; Hansen, 2009; Krishnan *et al*., 2017).In addition, TS was found associated with increased twinning rate due to co-dominance of sub-ordinate follicles and multiple ovulation, various teratology in post-implantation embryos (Wolfenson *et al.,* 2000; Pedersen, 2014) and foetal stress due to physiological aberrations which could affect their performance later in the adult-hood (Hansen, 2009).

Kristyna *et al*. (2017) reported that season of the year had significant influence on conception rate of HF dairy cows where higher conception rate was observed during winter (18.21 % more) than summer season and obtained highest conception rate during February (91.67 %), when temperature was below 5°C (1.42 ± 3.44 °C). Similarly, lowest conception (22.73 %) was in September, when AbTof the time of insemination was 16.94 ± 3.61°C. Conception rate maintained an inverse relationship with RT as evidenced from lower conception rate during spring and summer with higher RTwhen compared to autumn and winter (Sonmez *et al*., 2005; De-Souza *et al*., 2016). Similarly, cows inseminated during the morning hours when the RT was low achieved better conception rate than those inseminated in the afternoon (De-Souza *et al*., 2016).

In general, there was 4.6 per cent drop in the conception rate of AI, for each unit increment of THI beyond 70 (Krishnan *et al*., 2017). Daily exposure of dairy cows to TS (THI 72 or more) starting from Day 35 pre-serviceto Day 6 post-serviceresulted in a reduction of conception rate by around 30 per cent. However, when the THI was more than 80 from Day 3 to Day 1 prior to insemination, the conception rate decrease was from 30.6 to 23 per cent (Ispierto *et al,* 2007; El-Tarabany and El-Tarabany, 2015; Schuller *et al*. 2017). Dominant follicles exposed to TS failed to attain usual size and transformed into smaller sized CLcaused by low level of LH stimulation for luteinization and reduced P4 secretion (Wakayo *et al.*, 2015).

Thermal stress brings about increased level of circulating prolactin that lead to suspension of oestrous cycle and infertility (Alamer, 2011). Increased embryonic mortality rate (26 %) was observed in purebred HF cows than in BS cows orcrossbreds of HF and BS cows maintained at low, moderate and high THI, which indicated that high THI exposure caused considerable decline of conception rate in purebred HF than others (El-Tarabany and El-Bayoumi, 2015). Cows exposed to TS during the last three months of gestation suffered more peri-parturient complications (Bolocan, 2009). Maternal TS also caused impairments of placental function and affected foetal development, reduced secretion of placental hormones and reduced milk yield leading to inadequate nutrition of the neonate (Hansen, 2009).

2.5.8. Influence on Pregnancy Loss

Embryonic mortality comprised death of the conceptus prior to Day 42 of pregnancy and the incidence ranged from 10 to 40 per cent in initial breeders to 65 per cent in repeat-breeder cattle (Saacke *et al.*, 2000; Das *et al.*, 2016). Increased metabolism and liver perfusion rate in high producing dairy cows caused rapid elimination of ovarian steroids that ledto increased rate of pregnancy loss(Wiltbank *et al.*, 2006; Abdalla *et al.*, 2017). P4 concentration in cows which maintained pregnancy and those suffered embryonic mortality were 4.20 ± 1.40 ng/mL and 1.93 ± 0.30 ng/mL, respectively (Siregar *et al.*, 2017). In high producing cows, most (90 % or more) pregnancy losses were found to occur within 90 days of conception and more frequently before Day 50 since the placental development was yet to be completed (De Rensis, 2015).

The mechanisms by which TS contributes to early embryonic death (EED)involved interference of protein synthesis, direct effect through oxidative cell damage and also by reducing the production of IFN-τ involved in signaling for maternal recognition of pregnancy (Wolfensen *et al.*, 2000; Pedersen, 2014). Pregnancy loss could occur at different stages of pregnancy even though early embryos werehighly susceptible to environmental stressors(Abdalla *et al.*, 2017). The relative risk of embryonic loss in response to THI elevation was found to be considerably high between 21 to 30 days of conception (Ispierto *et al.*, 2006). As gestation advanced, there was proportionate reduction so that pregnancy wastage during the stage of foetus (after Day 42) was below 10 per cent (Goeseels and Kastelic, 2003).

Even though EED was often indicated by prolongation of the inter-oestrus interval, most of the embryonic losses occurring between Days 8 and 17 goes undetected (Thatcher *et al.*, 2001). However, Goeseels and Kastelic (2003) could assess the pregnancy loss between Days 8 and 17 as 24.4 per cent, by eliminating conception failures from lack of fertilization and embryonic mortality within the first week of conception,by transfer of good quality embryos into healthy recipient cows. Rather than direct effect, TS also affected the maternal physiology and caused intra uterine death of the embryos (Hansen, 2009). On post-implantation embryos and foetus, TS has been found to produce various teratologies as well (Wolfensen *et al*., 2000).

In purebred HF cows subjected to TS, foetal loss rate was significantly increased from 17.1 per cent at a lower level of THI to 24.9 per cent at higher THI. Correspondingly, there were increase in the rates of abortion (3.6 to 7.2 %) and stillbirth (3.8 to 5.9 %) for low and high THI exposure, respectively. On the contrary, there was not much difference in the foetal loss rateamong crossbreds of BS and HF at different levels of THI indicative of the breed difference (El-Tarabany and El-Tarabany, 2015).

Incidence of embryonic loss after the first AI was 31.6 and 14.7 per cent respectively during the early and late phases of embryonic development (Humblot, 2001). In high - producing cows, embryonic death after Day 27 of gestation was 3.2 per cent under normal climate and increased up to 42.7 per cent under TS prone climate (Cartmill *et al*., 2001; Silke *et al.*, 2002). Besides causing embryonic death as the immediate responseduring early pregnancy, delayed effect of TS was also evidenced (Ispierto *et al.*, 2006) since cows exposed to high THI between Days 21 to 30 of gestation showed more incidence of pregnancy loss extending up to 90 days of gestation (De-Rensis*et al.,* 2017).

2.6. THERMAL STRESS INDICATORS

Different environmental variables contribute to TS in animals and elevation of AbTforms the major one. BT of dairy cattle was found to be regulated by so many factors and hot weather together with higher RH played crucial role as determinants of TS (Kadzere *et al*., 2002). In addition, various weather parameters were found to affectphysiological processes and welfare of the animal either direclty and /or indireclty such as day length, solar radiation, rainfall and wind velocity (Dash *et al*., 2016; De-Rensis*et al.,* 2017). All these variables werefound to regulate TS through the influence onAbT and RH (Mader *et al*., 2006).

Combined effect of AbT and RH in producing TS was better represented by the THI (Polsky and Von- Keyserlingk, 2017). Since AbT and RH are the only variables considered for arriving THI, there is strong argument that additional factors also should be included in the calculation of TS indices wherever possible (Polsky and Von- Keyserlingk, 2017). In this respect, studying the seasonal trendwas suggested as a better way for understanding the influence of TS on mammalian reproduction since combined effect of more weather parameterscould be considered simultaneoulsy (Hansen, 2009).

2.6.1. Temperature Humidity Index (THI)

THI formed a combined index of AbT and RH arrived using diffferent formulae and having no unit. THI was first introduced by Thom (1959) in human beings and later adopted to describe TS conditions in animals as well (De-Rensis*et al*., 2015). Calculation of TS due to environmental adversities depended on which formula was chosen. Each of the THI equations was giving different weightage for humidity and dry-bulb temperature to account for different environmental conditions (Bohmanova *et al*., 2007). According to Gaughan *et al*. (2018) the THI was limiting as it did not includecertain important climaticvariables and emphasized the need to include wind speed,solar radiation, shade provision and animal factors such as breed. Even then, the categorical THI was found to be useful as a rough indicator of the effects of TS on body systems of the animal (Bohmanova *et al*., 2007).

The THI was categorized into different levels to denote the extent of TS exposed to the individual, but definitions vary between researchers and conditions. In general THI of <72 was considered as thermal comfort zone and THI of 72 and above was found to cause different levels of stress (Armstrong, 1994). THI levels in the ranges between 72 to 79, 80 to 89 and 90 and above were described to cause mild, moderate and severe stress respectively(Dash *et al*., 2016; Archana *et al*., 2017;Polsky and Von-Keyserlingk, 2017). But recently, lowering the bottom limit of thermal comfort zone to 68 has been suggested especially for high yielding dairy cows which were highly stress prone and mild signs of TS was shown by these animalswithin the THI range of 68 to 71 (Zimbelman *et al.*, 2009; De-Rensis*et al*., 2015; Krishnan *et al*., 2017).

The usefulness of THI as an indicator of TS was dependent on so many factors such as genotype of the animal, breed and productivity, age and parity and management situations. In dairy animals exposed to TS, drastic reduction of milk production was observed whenever the THI exceeded 74 (Pedersen, 2014; Polsky and Von-Keyserlingk, 2017). The sensitivity to TS increasedtogether with the hike of milk production. A rise in daily milk yield of cows from 35 to 45 litres/dayhas been found to reduce the threshold temperature limit for TS by 5°C (Berman, 2005). Similarly, pluriparous cows were found more susceptible to TS and a reduction in their milk yield as much as 1 kg/day with each increment of THI beyond 72 was observed by Bernabucci *et al.* (2014).

Milk production of cows was found affected as the immediate response to TS, while fertility was also affected seriously which could be observed only later(Krishnan *et al.*, 2017). Whenever THI exceeded 72, there was corresponding decrease in the rates of conception as well as resultant pregnancies thereafter(Dash *et al.*, 2016). PureHF cows were more sensitive to TS than other breeds, as reflected by more incidences of foetal lossand pregnancy rate decline at high THI (El-Tarabany and El-Tarabany, 2015). Besides production loss, exposure of HF cows to high THI caused significant reduction of pregnancy rates in high producing animals (Abdalla *et al.*, 2017; Guo *et al.*, 2018).In addition, higher incidence of foetal loss, abortion, still birth and calving difficulties, long inter calving period, and long open days were also detected in high producers, where as no significant changes of any of these parameters were observed in low producing dairy breeds as well as crossbreds even at high THI (El-Tarabany and El-Tarabany, 2015).

Krishnan *et al.*(2017) reported that in dairy cows for each unit of THI elevation above 70, conception rate was reduced by 4.6 per cent.At low THI, fertility rates were more or less same for different genotypes indicated by similar conception rate, pregnancy proportion and embryonic loss rates for purebred and crossbred HF cows.Morton *et al.* (2007) estimated that exposure of lactating dairy cows to TS (daily maximum THI $\geq$72)from35 daysbefore until six days after AI, reduced the conception rate by 30 per cent. Furthermore, exposure to maximum THI of$\geq$80 from the proestrus onwards decreased the conception rate from 30.6 to 23 per cent (Ispierto *et al.*, 2007).

The THI is considered as an indicator of TS more suited to dairy cows as well as milch baffaloes. In cattle, the milk production decreased drastically whenever, THI exceeded 72 (Guo *et al.*, 2018). At the same time, buffaloes were less sensitive to TS than high yielding cattle. Fertility of buffaloes was found to decrease considerably following exposure to THI exceeding 75, whereas the reduction was much less below THI 75 compared to cattle (Dash *et al.*, 2016; Krishnan *et al.*, 2017).

Heat stress conditions as indicated by increased THI have been shown to influence the core BT, standing behaviour and milk production (Allen *et al.*, 2015). In cattle, THI elevation beyond 72 was reported to cause suppression of oestrus signs including colour of vaginal mucosa, mounting activity, oestrus discharge, follicle diameter and serum P4 concentration. Ultimately the conception rate was reduced continuously and maintained an inverse relationship with THI elevation beyond 72 (Schuller *et al.*, 2017).

Wolfenson *et al.* (2000)noted that high THI during summer affected endometrial function, altering its secretory activity, related hormonal functions and reducing even the mucous flow of the oestrus. Increase of THI more than 74 resulted continuous reduction of serum P4 level, size of oestrus follicle and intensity of external oestrus signs. However, no significant association was found between THI value and variables such as occurrence of ovarian cysts and uterine contractility even though uterine blood flow was seriously affected (Schuller *et al.*, 2017).

2.6.2. Heat Shock Protein – 70 (HSP -70)

Elevation of AbTbeyond the normal limit of BT was found to cause TS so that normal homeostasis of the body gets disturbed. TS caused expression of many proteins in the system and HSPs are the major group that played crucial role to ameliorate the deleterious effects of TS (Mishra and Palai, 2014; Rajoriya *et al*., 2014). HSPswere designated by their molecular weight in Kilodaltons as HSP 70, HSP 90 and so on (Marruchella *et al*., 2004). Exposure to stress was associated with HSP production mainly from the gut, liver and other tissues (Mapham and Vorster, 2013) and released in an inducible form into intra-cellular and extra-cellularlocations(Doklandy *et al*., 2006; Hecker and McGarvey, 2011) intended for the repair of cellular damage in different parts of the body (Gaughan, *et al.*, 2013).

Elevation of HSP 70 and HSP 90 were observed in most of the farm animals such as cattle, buffalo, sheep, goat and broilers (Shaji *et al*., 2015).Elevationof temperature, physical strain, ischemia and oxidative stress are some conditions inducing HSP synthesis(Iwaki *et al*., 1993).In addition, activation of HSPs in response to bacterial invasion was found to stimulate the immune system to mobilize neutrophils, macrophages and other innate immune cells to effect elimination of pathogenic bacteria (Mapham and Vorster, 2013; Archana *et al*., 2017).Embryonic tissue also produced HSP among the various proteins produced during its development and was found to perform important cellular functions starting from embryonic life (De-Rensis *et al.*, 2017).

Among the HSPs, HSP70i (namely, HSP70.1 and HSP70.2) was found to be the most temperature sensitive one and its production was initiated by various environmental stressors and pathological deviations of the body (Beckham *et al*., 2004; Maibam *et al*., 2017). HSP 70i is the inducible form of HSP70 and has been suggested as an indicator of thermo-tolerance in livestock species (Romero *et al*., 2013). Maibam *et al*. (2017) reported elevation in the expression of genes for constitutive (HSP 70.8) and inducible (HSP70.1, HSP70.2) forms of HSP with an increased magnitude during summer than winter (Maibam *et al*., 2017). However, Rajoriya *et al*. (2014) found no significant difference in mRNA expression of HSP 70 and HSP 90 in the semen of Tharparkar bull during winter and summer seasons and attributed the same to stress resistance of the breed.

Function of HSPs were mainly as molecular chaperones to repair the cellular damages consequent to TS, thus to restore the cellular homeostasis and to enhance the cell survival.Such cellular repair mechanism mainly involved corrective folding, unfolding and refolding of denatured proteins (Collier *et al*., 2008; Rajib *et al*., 2015). HSPs were also involved in preventing cellular apoptosis and restoration of cellular functions (Mishra and Palai, 2014; Archana *et al*., 2017). Further, increased expression of one or more HSP genes enabledthe cells to resist further damage and to ensure protection of the cell against stressors occurring even afterwards (Luh *et al*., 2007; Mapham and Vorster, 2013).

Heat shock proteins were found to perform important cellular roles in order to ensure tolerance against environmental stress and to achieve adaptation to TS (Kumar *et al*., 2015). Being highly conserved proteins, HSP s werefound to be easily activated by heat and other stressors including thermal and oxidative stress (Hecker and McGarvey, 2011; Archana *et al*., 2017). The induction and synthesis of HSPs occurredintensivelyand rapidly as an emergency cellular response and the severity of stress was involved in regulating the intensity of HSP expression(Feder and Hoffman, 1999). Among various HSPs, HSP 70 were having important roles in deciding the tolerance and adaptation of the animal to TS and other hostile environmental conditions (King *et al*., 2002; Rajoriya *et al*., 2014; Maibam *et al*., 2017). In pregnant animals,HSPshave also been found to improve embryonic survival and reproductive efficiency (Archana *et al*., 2017).

Mosser *et al*. (1987) reported significant increase in the expression of HSP70 genes not only during summer, but also during winter season in skin of Karan Fries and Tharparkar cattle. Further, based on the observation of reduced development of thermo tolerance consequent to inhibition of HSP synthesis, they inferred that HSPs were important factors for theadaptation to seasonal variations of AbT(Maibam *et al*., 2017). In Murrah buffalo calves exposed to TS conditions, Mishra *et al*., (2011) found nearly 200 times increase in serum HSP70 levels. Zarina (2016) also had similar findings. Hence HSP 70 was found to be a potential marker for TS as opined by Patir and Upadhyay (2010), Kumar *et al*. (2015) and Archana *et al*. (2017).

Expression of thermo-tolerant genes and elevation of HSPs were the mechanisms by which the cells survive TS (Archana *et al.*, 2017). The expression of HSP genes were transcriptionally regulated (Rajoriya *et al.*, 2014) and the biological response to TS in mammals was controlled at the transcription level through the activation of HSF (heat shock factor) genes. Elevation of HSF activatedthe transcription of mRNA for production of HSPs such as HSP 70, HSP 90 and HSP 27. Among these, HSP 70 was found to be most important one in regulating TS in animals (Beckham *et al*., 2004; Archana *et al*., 2017). Gaughan *et al.* (2013) reported a strong relationship of HSP 70 concentration with AbTand photoperiod and concluded that HSP 70 concentration formed an important indicator of chronic stress especially in animalssubjected to multiple and persistent stress factors.

2.6.3. Serum Cortisol

Thermal stress has been found to affect the hypothalamo hypophyseal gonadal axis directly and is manifested through alterations in the amount of cortisol - the major stress hormone in case of ruminants (Binsiya *et al*., 2017; De-Rensis*et al.*, 2017). While acute TS caused increased secretion of cortisol (Rees *et al*. 2016; Marina and Von Keyserlingk, 2017), the level was found to be reduced in cows under chronic TS (Silanikove, 2000), and was often associated with reduced fertility (Maciel *et al*., 2001). Ronchi *et al*. (2001) reported significant elevation of cortisol level during pro-oestrus and oestrus in animals housed in a thermal chamber as well as those exposed to high air temperature and inferred that while chronic TS depressed cortisol concentrations outside the follicular phase, there was no alteration in the mechanism responsible for increased cortisol secretion of follicular phase.

Torres-Junior *et al*. (2008) observed thatcortisol secretion was influenced by management conditions since the levels were high in cows managed under tied in stall system than those kept on pasture. However, feed restriction was not found to have any effects on cortisol secretion, since high cortisol levels during summer was due to the direct effect of TS rather than indirect mechanisms acting through reduced feed intake (Ronchi *et al*., 2001). Shaji *et al.* (2015) reported an up regulation of HSP70 expression and increased synthesis of cortisol associated with hyperactivity of adrenal cortex during TS.

Cortisol level was found to be significantly low in sheep subjected to multiple stressors such as heat, increased walking and nutritional alterations (Sejian *et al*., 2013a). Alameen and Abdelatif (2012) observed higher cortisol concentration in crossbred cattle during summer compared to those in winter. Bhan *et al*. (2012) observed significant increase in the concentration of cortisol in Sahiwal cattle starting from 1.92 to 8.91 ng/mL simultaneous with the increase of THI from 62.0 to 79.0. Bouraoui *et al*. (2002) observed higher levels of cortisol in lactating HF cows during summer (THI = 78) compared to spring (THI = 68) and also there was a positive correlation between cortisol levels and THI.

Wanker *et al*. (2014) reported significant rise incortisol levels with elevation of AbTfrom 25 to 35^{o}C compared to 25^{o}C, while there was no significant difference in the levels of cortisol at 30^{o}C and 40^{o}C in adult buffaloes. Nikhil (2015) also reported non-significant difference in the concentration of plasma cortisol levels in crossbred calves during pre-monsoon, monsoon and post monsoon seasons.Even though young and adult buffaloes exhibited significantly high levels of cortisol at 40, 42 and 45^{o}C compared to 22^{o}C, the levels did not differ significantly between 40^{o}C, 42^{o}C and 45^{o}C (Haque *et al*., 2012).They explained the significant elevation of cortisol levels being caused by the activation of HPA, thus enhancing the adaptability of animals during the stress period.

Prasad (2014) did not find any statistical difference in the average plasma cortisol values in cows from different THI zones of Kerala. He also measured circulating plasma cortisol values in animals under different types of TS alleviation measures and no significant difference could be observed. Zarina (2016) reported no significant correlation of serum cortisol level with THI in buffalo calves and crossbred cattle calves at the same location. However, Harikumar (2017) reported significant elevation of cortisol levels in TS exposed dairy cows indicated by high THI values, and suggested cortisol level estimation as a useful indicator to assess stress response in animals.

2.6.4. Antioxidant Levels in Serum:

Oxidative damage consequent to TS caused increased production of reactive oxygen species (ROS) in different cells and tissues, which in turn produced various adverse effects on normal physiology and body metabolism (Das *et al*., 2016). In response or as a preventive measure, there was mobilization of different enzymatic and non-enzymatic antioxidants in the body including malondialdehyde (MDA), which were acting to counteract the effects of ROS in stress exposed animals (Lallawmkimi, *et al*., 2013; Yatoo *et al*., 2014).

Many deleterious effects of elevated AbT on gametes and embryos were caused through the mediation of ROS (Hansen, 2009). Thermal stress was found to produce damage to the oocyte during the pre-ovulatory period and pre-implantation embryos, and was mediated through the generation of ROS (Roth *et al*., 2000; De-Rensis*et al.,* 2017). Also such damages caused by ROS were found to be reduced *in vitro* by the administration of antioxidants (Lawrence *et al*., 2004). A study by Guo *et al*. (2018) showed increased level of MDA and reduced activity of glutathione peroxidase in cows under TS and was caused by increased lipid peroxidation of hyperthermic environment that suppressed the activity of antioxidant enzymes (Zuo *et al*., 2000).

Activities of antioxidant enzymes were inhibited by excessive accumulation of MDA leading to impairment of antioxidative ability(Guo *et al*., 2018). Oxidative stress also affected steroidogenesis by follicular and/or luteal cells. Peroxidation capacity of liver and activity of enzymes involved in ROS production such as cyclo-oxygenase and xanthine oxidase were increased by elevation of AbT. Further, total antioxidant activity in blood was reduced leading to increased production of ROS and affected P4secretion by causing impairment of LH receptors (Torres-Junior *et al*., 2008).

Cell organelles damaged directly or indirectly through oxidative stress have been found to cause DNA damage (leadto apoptosis), reduced developmental competence and altered gene expression in pre-implantation embryos. Oocytes, zygotes, and early stage embryos were less capable of resisting ROS emissions (Sakatani, 2017) and enhancement of anti-oxidant capacitywas shown enhanced through supplementation of suitable antioxidants so that improvement of oocyte and embryo quality and improved developmental competence could be achieved under TS (Sakatani,2017).

Highconcentration of thiobarbituric acid reactive substance (TBARS) has been reported in dairy cows during hot weather compared to coolerenvironment (Sakatani *et al.,* 2012b). Significantly higher levels of stress indices such as catalase, Glutathione (GSH) reductase and MDA was observed during summer compared to spring seasons in lactating and non-lactating buffaloes (Lallawmkimi *et al*., 2013; Yatoo *et al*., 2014). Besides climate, lactation also contributed significantly high levels of stress and increased milk production enhanced TS-induced oxidative damages in the body (Yatoo *et al*., 2014; Das *et al*., 2016).

2.7. ADAPTATION TO THERMAL STRESS

Homeotherms are animals having the ability to regulate their BT in spite of variation in AbT, which is an evolutionary capacity that has made these animals to survive and maintain homeostasis and normal bodily activities under wide variations of climate (Kadzere *et al*., 2002). The animals showed various physiological adaptation mechanisms to cope up with the adverse climate, which included increase of RR, RT, pulse rate, skin temperature and sweating. All these formedthe natural mechanisms helping to expel the extra heat load in an effort to maintain the homeostasis (Indu and Pareek, 2015; El- Tarabany *et al.,* 2017). These physiological parameters increased with TS and were considered as the physiological determinants of adaptations to TS (Singh *et al.*, 2016; Rashamol *et al.,* 2018).

In addition to the physiologicalcoping strategiesinvolved in the regulation of BT as an immediate measure, certain behavioural alterations were also initiated such as modified pattern of drinking and feed intake, increased standing time, shade seeking, decreased activity and transient reduction in milk yield (West, 2003). In addition, there was certain long term adaptational changes associated with TS such as decreased lactation yield and reproductive performance (De-Rensis and Scaramuzzi, 2003;Polsky and Von Keyserlingk, 2017).

The adaptive capacity of the animals varied between species, breeds, strains, productivity levels and so on. Adaptation differences between animals were determined by the quantum of TS and the duration of such exposure (Kadzere *et al.*, 2002; El- Tarabany *et al.,* 2017). Indigenous breeds were having higher thermo-tolerant capacity when compared to exotic animals, and also crossbreds have better thermo tolerance than purebreds (Von Keyserlingk *et al.*, 2013; Archana *et al.*, 2017; Maibam *et al*., 2017; Rashamol *et al.,* 2018).

Genetic selection of animals for their thermo-tolerance was one of the important steps towards reducing the impact of TS on dairy cattle. Genetic differences between different types of animals were made possible by thermo-tolerance at the cellular levels produced by the family of celluar proteins *viz*HSP (King *et al.*, 2002; Rajib *et al.*, 2015). Among the HSP family of proteins, HSP70 was the major one involved in thermo-tolerance and contributing towards animal survival under climatic adversities (Beckham *et al.*, 2004; Jonak *et al.,* 2006; Archana *et al.,* 2017).

Synthesis and release of HSP formed a generalized mechanism of coping with TS present in all cells, which also has got regulatory roles in various types of immunity. The capacity of each animal to produce HSP and thus to cope up with the stress varied depending upon various factors (Archana *et al,* 2017). Significant upregulation of HSP 70 genes was observedduring summer and winter season in the skin of Zebu cattle than their crossbreds that helped to provide increased protection against TS during hot weather conditions (Mosser *et al.*, 1987). Such an increase was considered important for adaptation of these animals to seasonal variation of AbT, since inhibition of HSP synthesis was found to prevent the development of thermo-tolerance in such breeds (Mosser *et al*., 1987; Maibam *et al*., 2017).

Thermal stress was found to cause a significant decline in reproductive performance of buffaloes when the THI exceeded above the threshold level of 75. Hence, inclusion of the response to THI elevation as a selection criterion had been suggested to improve the adaptability of dairy animals in the hot climate (Dash *et al*., 2016). Genetic adaptation to TS was made possible in two ways such as regulation of the BT to the normal range by physiological and behavioural alterations and enhancing resistance to elevated temperature at cellular level(Hansen, 2009; Archana *et al*, 2017). In regions of consistently moderate to high AbT, promotion of breeds with better adaptation to higher temperatures has been advised in order to reduce the harmful effects of TS (Von-Keyserlingk *et al*., 2013).

Alterations in reproductive performance consequent to prolonged TS exposure was found to cause variations in ovarian activity and oestrus behavior of ewes leading to reduction of fertility as well as productivity of the flock. Also there were genetic differences among sheep breeds with respect to heat tolerance which was found to have negative relationship with reproductive performance (Kadzere *et al*., 2002). However, different breeds vary with respect to their adaptability to high environmental temperatures without much variation in their oestrus activity (Macias-Cruz *et al*., 2016).

There were some studies documenting the need for relatively long time for adaptation to the negative effect of high AbT and to restore normal level of fertility for dairy cows with high production or reproduction performance (Kristyna *et al*., 2017). Accordingly, *Bos taurus*breeds, owing to their higher productivity are at high risk for TS and need more time for adaptation compared to *B. indicus* cattle. This was mainly attributed to their lack of functional evolutionary adaptations to tolerate higher AbT (Von Keyserlingk *et al*., 2013).

Adaptation to prolonged TS invariably leads to reduced production capacity (Sejian *et al.*, 2010) as well as reproductive performance (De-Rensis and Scaramuzzi, 2003. Polsky and Von Keyserlingk, 2017).This was occurring as the consequence of adaptive and associated compensatory mechanisms to maintain homeostasis that favour survival in a consistently hostile environment (Indu and Pareek, 2015).Exploring the physiological adaptive mechanisms of the animal in detail and identifying suitable breeds or strains well adapted to the agro climatic zones (Rashamol *et al*., 2018) can be the major step to ensure climate resilient livestock production in the context of progressive global warming.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

a. Study Location

The study was carried out at the dairy farm of Livestock Research Station (LRS), Thiruvazhamkunnu in Palakkad District of Kerala. The farm belongs to Kerala Veterinary and Animal Sciences University holding mainly of dairy cattle. The station is having an area of 163 hectares constituted of a dense strip of natural forest forming almost half of the land area, which is lying in proximity to the ever green forest of Western Ghats.

Geographically, the farm is situated at an altitude of 60-70 meters above mean sea level, with latitude and longitude positioning denoted by 11°21' N and 76°21' E, respectively (Anoop *et al*., 2011). Bare rocks of the nearby mountains get heated up faster and emit thermal radiations throughout the day in most part of the year, which in turn causes cumulative elevation of AbTexposing the animals to stress prone conditions. Hence, the dairy farm of the station was selected for the study to assess the influence of TS.

b. Climatic Features

The maximum temperature (MxT) of the locality reaches upto 40°C some days, while minimum temperature (MnT) of the area is beyond 15 °C even during the colder season. The diurnal variation of the AbThas been found ranged from 15–30 °C during colder season and 25 to 40 °C during summer. Average annual rainfall was 700 mm, mostly showered from early June to mid-September. The mean RH of the region was very high not only during the rainy season(from June to November, comprised of south west and north east monsoons) but also during most part of the year even reaching saturation many days.

c. Classification of Seasons

Classification of the seasons as four quarters of three months each was followed similar to an earlier study on seasonal fertility of goats in Kerala (Kutty, 1995). This classification was preferred as it considered not only AtT and raining but day length as well, as the main regulators of season and hence used as the operational classification in this study as well. The four seasons thus designated in the study were North East monsoon (NEM) comprised of September-October-November (SON) Post-monsoon (PMN) included months of comparatively colder weather*viz.* December - January - February (DJF), Summer (SUM) with warmest three months such as March – April – May (MAM) and South West monsoon (SWM) included three months *viz*. June-July-August (JJA) characterized by heavy raining.

Plate 1.a. Cattle barn of LRS where the study was carried out

Plate 1.b. Study animals housed inside the barn

d. Animal Stock,Housing and Management

About 250 heads of crossbred cattle of the farm were progenies of local non-descript animals with exotic breeds such as Jersey, HF and BS (Plate 1.b). Considering the prevailing warm climate, Jersey breed is given preference in the current breeding strategy of the farm, to enhance the productivity, climatic adaptability and management convenience of crossbred dairy cattle.

Adult cows of the farm were, maintained intensively in barns (Plate 1.a.) built with open sides and AC (asbestos – cement) sheet roofing, with a maximum height of 5 meters at the centre and sloping to 3 meters on either sides. Each barn constructed in north south direction, houses 40 - 50 animals in tail to tail manner. While dry animals were sent out for grazing during summer months for about 4-5 hours, milking animals were retained in barns and provided with green fodder throughout the milking period. Other details of feeding and routine management of the study animals are described later. Oestrus was detected by human observation and breeding was exclusively through AI using frozen semen straws purchased from Kerala Livestock Development Board.

e. Plan of Study

The study was planned with components of retrospective data collection for a period of six years and prospective study with regular and frequent data collection on various general and fertility related parameters over the period of one year from September 2018 to August 2019.Major climatic factors causing TSsuch as variants of AbT and RH were recorded and THI was calculated on a daily basis. Influence of theclimatic stress factors on the study parameters were assessed on individual basis and combined influence was assessed based on the seasonal pattern. Accordingly, the period of study was divided into four seasons as mentioned before, considering climatic features of the locality and complying with established classification of seasons in Kerala (Kumar 2013; Kutty 2013; Rao 2013).

3.1. RETROSPECTIVE STUDY

Retrospective data were collected from breeding records and stock details maintained at the farm. Breeding related details of all the cows available during the six year period(September 2013 to August 2019) were collected, excluding those marked to have diagnosed Brucellosis or Tuberculosisduring the period of data collection.The reproductive parameters of the retrospective period studied were:

- Number of breedable cows
- Nmber of oestrus detected
- Number of AI done
- First PP oestrus to conception (Serviceperiod)
- Services per conception
- Conception rate of AI
- Double AI conception
- Calving-concep. Interval (Days open)
- Inter calving period
- Herd pregnancy rate
- Pregnancy loss or abortions
- Number of calvings per month

3.1.1. Weather Parameters in the Past Six Years

Weather parameters in the past six years were collected from the Automatic Weather Station (Campbell Scientific, CR 800 series data logger) situated at the station(Plate 2.a.)andtwo other weather stations located at VFPCK Karimpuzha and Elavanchery, which were situated within 25 Kilometers distance from the farm. From the hourly recordings of AbT and RH, weather parameters such as daily average temperature (AvT), average relative humidity (AvRH), MxT, MnT, maximum relative humidity (MxRH) and minimum relative humidity (MnRH) were collected. THI values were calculated from AvT and AvRH using the formula for livestock and poultry heat stress index (LPHSI,1990),intended for demarcation of the study period based on zone of comfort. Weather parameters and fertility parameters were compared to study the influence of TS during the period of six years.

LPHSI is given by the formula:

$$THI\ (LPHSI) = T - \left(\left(0.55 - \frac{0.55 \times RH}{100}\right) \times (T - 58)\right)$$

Where T - Average temperature (in Degree Fahrenheit)
RH - Per cent relative humidity

3.2. PROSPECTIVE STUDY (Effect of TS on Physiological and Reproductive Parameters).

Prospective data were collected during the period of one year from September 2018 onwards. Every month general study parameters were observed between Day 7 to Day 135 PPin 22 cows (Group 1 and Group 2). Detailed investigations including recording of RR and RT, ultrasonography (USG) studies, and estimation of stress indicators and reproductive hormones were carried out every week,starting from Day 28 to Day 91 PP,in eight animals (randomly selected from the total 22 cows). All the cows under the study each month (n=22) constituted Group 1, and 8 cows selected for the detailed study each month (out of the 22)were designated as Group2, respectively.

3.2.1. Recording of weather parameters

Daily ambient conditions were collected with Hobo data logger (HOBO pro V2, Onset Computer Corporation, USA) fixed within the barn (Fig. 2.b.) in addition to macro climate recording available from the automatic weather station situated 50 meters away from the study animal housing. The data logger was set for hourly recording of AbT and RH and daily AvT and AvRH were downloaded periodically and stored for analysis.

3.2.1.1.Temperature Humidity Index

Daily average values of AbT and RH were used for calculation of THI using the same formula (LPHSI) mentioned under the section 3.1.1.

3.2.1.2. ***Other Climatic Stress Factors***

Recordings of MxT, MnT, MxRH and MnRH were also collected from the routine hourly recordings of temperature and humidity with respective times of attainment from both automatic weather station (macro climate) as well as Hobo Data logger (micro climate).

3.2.2. Seasonality of Thermal Stress

Periodic trends of weather data collected under the retrospective and prospective study periods including THI calculated and other data from meteorological station were compared for any similarity of the trends.All the study parameters were then compared with the operational classification of the seasons to understand the seasonal pattern and variability together with the influence of weatherparameters or THI and HSP 70 across seasons.

3.2.3. Thermal Stress Influence on Dairy Cattle

Influence of TS on the animal was assessed by changes in the general physiological parameters such as feeding, milk production and BCS, and reproductive performance parameters. Physiological indicators of stress in the circulation were estimated, monthly and seasonal pattern of stress indicators, weather parameters and other study parameters were compared and correlation between all these parameters were also assessed.

3.2.3.1.Stress Indicators

Frozen (-20 °C) stored serum samples collected and stored during the course of study (details under sub section 3.2.10) weresubjected to enzyme linked immune-sorbent assay (ELISA) for HSP 70 and cortisol using specific ELISA Kits(sub section 3.2.10.1 and 3.2.10.2.). Detailed steps of ELISA for these two parameters are given under Appendices 5

Plate 2.a. Automatic weather station installed in the farm

Plate 2.b. Hobo data logger installed in the animal shed

and 6. Levels of stress indicators thus assessed were compared with various study parameters to understand the nature and severity of TS influence.

3.2.4. Selection of Cows for the Study

Seven apparently healthy postpartum (PP)cows without any peri-partum complications and second to fifth parity were enrolled every month starting from July 2018 based on the sequence of calving date. Animals with very poor (< 2.5) and very high (>3.5) BCS and very irregular milk yield in the previous lactation were not included for the study. The study group consisted of 22 cows (n=22) from 7 to 135 days PP were housed together throughout the study period. Every month new enrolments of suitable cows were made replacing earlier ones and those completed at least 91 days under the study.

3.2.5. Management

Uniform management was provided for all the cows based on the health and production status. Clean drinking water was made available always using automatic watering facility fitted in front of each cow. Oscillating wall fans were provided in the barns and were operated during hot weather. Total body washing of the cows was performed routinely once in the morning and partial washing of the hind quarter was carried out before milking.

Good quality green fodder (Hybrid Napier varieties) was provided *ad libitum*throughout the year and compounded cattle feed (CP – 19 % and TDN - 72.4%) was provided twice daily during the milking time measuring the quantity as per standard recommendations (ICAR-NIANP, 2013). Machine milking was practiced twice daily at 4.30 – 5.30 am and 12.30 - 1.30 pm. Breeding was exclusively through AI, observing a voluntary waiting period of 40 days. Detection of oestrus was performed by intermittent (within an interval of 6 h) human observation of signs exhibited by the animals restrained in barns and no hormonal induction of oestrus was performed throughout the study period.

3.2.6. Collection of General Data

General physical and management related parameters were observed, from 7 days PP and continued for 91 days or until replacement by newly enrolled cows, such as

3.2.6.1.Dry Matter Intake

Quantity of compounded cattle feed and fodder fed dailyto each cow was recorded. The quantity requirement was computed considering maintenance, milk yield and breeding status and was revised every week. The allocation was offered individually and left over /wastage, if any, was deducted to assess the actual intake. Major variations in quantity or quality of fodder provided were also noted and the DMI was assessed on a daily basis.

3.2.6.2.Milk Production

Milk yield at morning and noon milking rounded to half a liter was recorded and added to get the daily milk production and was continued throughout the study period.

3.2.6.3.Body Condition Score

Body condition score was recorded at weekly intervals based on a 1 to 5 scale with increments of 0.5 as per the method suggested for crossbred cattle by Smijisha (2012). In this method the animals were observed for the changes at seven aspects of the body, comparative scores were assigned and the final score was recorded by taking the average. The eight points of observation included:

1. Spinous processes on the loin region / Lumbar vertebra
2. Between spinous and transverse processes of the lumbar vertebra
3. Transverse process of the lumbar vertebra viewed near the flank
4. Overhanging shelf beneath the spines of the lumbar vertebra
5. Between pin bone (point of ischium) and hooks (external angle of ilium)
6. Between pin bone and sacrum (sacro sciatic fossa)
7. Prominence of the sacral crest (at croup region)
8. Prominence of the tail head (demarcations of the coccygeal vertebra)

Based on the final score, animals with a score of less than 2.5, 2.5 to 3.5 and more than 3.5 were classified under low, medium and high BCS groups.

3.2.7. Reproductive Management

All the animals were routinely observed for resumption of postpartum cyclical activity and breeding was carried out after a voluntary waiting period of 40 days.

3.2.7.1. Detection of Oestrus

All the animals were observed for oestrus signs frequently (at least for 10 minutes at 6 hours interval) and those having primary or secondary signs of oestrus were subjected to further verification of external changes and clinico-gynecological examination for confirmation of the oestrus based on internal changes.

3.2.7.2. Intensity of Oestrus

Grading of oestrus intensity as suggested by Van-Eerdenburg *et al* (1996) could not be adopted since the animals were confined to the barns all the time and lack opportunity for expressing primary signs of oestrus such as mounting and standing to be mounted. Hence oestrus scoring methods used by Azeez (2014) and Schuller *et al.* (2017) were adopted in this study with suitable modifications. Method of validation, criteria for scoring and model of detailed scoring sheet are shown in Appendix 2 and 3.

Animals were observed periodically and adequate scores were assigned for history, secondary behavioural signs, minor external manifestations and findings of clinico-gynecological examination. Those cows getting a score 40 or more out of 100 were considered for performing AI.

3.2.7.3. Insemination

Cows confirmed to be in oestrus based on the intensity score and devoid of any structural or functional abnormalities were inseminated using frozen-thawed semen. All the changes of oestrus such as behavioural features, external manifestations and internal changes were recorded. Inseminated animals were observed on subsequent days as well for the persistence of oestrus changes. Examination, recording of the findings and insemination were repeated for those continued to be in oestrus on subsequent days as well.

3.2.8. Detailed study– (Effect of TS on Physiological and Reproductive Performance of Crossbred Dairy Cows)**.**

Out of 22 animals under the study, 8 cows were subjected every month to observation of physiological and reproductive performance as well as ovarian status as shown in Table 3.1. Recording of physiologic parameters such as RR and RT,clinico-gynaecologic examination, USG of internal reproductive organs and blood sampling for estimating hormonal profile, heat stress indicators and anti-oxidant level in the serum were carried out at regular weekly intervals. Ultra sound scanning for ovarian structures and blood sampling were also carried out during oestrus. In addition,post-service blood samples were collected at weekly intervals from inseminated cows,and those not returned to oestrus were subjected to early pregnancy diagnosis using USG at 10 day intervals from day 25 post-AI.

3.2.8.1. Selection of Cows for the Detailed Study

Random selection of 4 animals was made every month and the study interventions were performed at weekly interval starting from day 28 to 91 PP. Listing of animals for each day's investigation based on their date of calving was obtained from an Excel based computer programme for easy management (Appendix No. 4.) and the details of investigations were recorded with proper codes for identification of the blood samples, scanning events and other parameters.

Table 3.1.Prospective study parameters and data collection frequency

Study parameters	*Data collection frequency*
Weather parameters during the study period	Daily recording
Details of feeding and milk yield (n=22)	Daily observation
Body condition score (n=22)	7 days interval
Oestrus characteristics (n=22)	Routine
AI at oestrus (after confirmation), (n=22)	Timely
Pregnancy Diagnosis (among non-returns), (n=22)	Day 45, 60 and 90 post AI
Body temperature and respiratory rate (n=8)	7 days interval during 6 months
Resumption of PP cycle by ultrasonography (n=8)	7 days interval from 28 days PP
Emergence of follicles by ultrasonography (n=8)	7 days interval from 28 days PP
Progesterone level of serum (n=8)	7 days interval from 28 days PP
Post-service progesterone level (n=8)	Days 7, 14 and 21 post oestrus
Oestradiol level during oestrus (n=8)	Days 0 and 1 of oestrus
Pregnancy loss among those conceived, using USG (n=8)	Days 25, 35, 45Post AI
Blood sampling for HSP 70 and cortisol (n=8)	7 days interval during 12 months
Blood sampling for Malondialdehyde (n=8)	7 days interval during 6 months

3.2.8.2. Respiration Rate and Rectal Temperature

Physiologic parameters such as RR and RT of the eight animalswere recorded six times during daytime (at three hours interval) and were repeated at 7 days intervals during the two quarters of low (DJF) and high THI (MAM). RR was recorded as the number of breaths per minute by counting the flank movements and RT was recorded using digital thermometer (Omron model MC 246®, Omron health care, Japan).

3.2.9. Ultrasonography

Ultrasound scanning of reproductive tract and ovaries were performed using Esaote Veterinary scanner (Mylab Delta model®, Esaote S.p.A, Italy), fitted with trans-rectal probe (5 MHz, SV 3513®) suitable for B mode, M mode and Color Doppler imaging (Plate 4.a.) to study the growth of follicles and CL. The animals were scanned at their standing space minimizing the stress of handling and translocation to any restraining facility. This was made possible by a movable trevis (Kutty *et al.* 2019) designed for the purpose (Plate 3.a., 3.b.).

Scanning operations were carried out throughout the study period (September 2018 to August 2019) at regular weekly intervals on 8 animals each starting from Day 28 to 91 PP. Inseminated animals were excluded from routine weekly scanning until return to oestrus or scanning for pregnancy in case of non-return animals. Animals returned to oestrus within 25 days or those found non-pregnant at scanning were re-enrolled for routine weekly scanning and blood collection among others not inseminated.

3.2.9.1.*Growth of Ovarian Follicles*

Follicles were identified as non-echogenic structures with a defined border between the follicle wall and antrum (Plate 4.b). Corpora lutea were identified as grainy echogenic structures with a distinct demarcation from the less echogenic normal ovarian stroma (Morris *et al*., 2011).Diameter of the follicles and corpora lutea was assessed as the average of the largest diameter taken in more than one direction.

Largest follicles were those with an internal diameter 9 mm or more in the absence of other actively growing follicles. Number and diameter of medium (6-8 mm), large (9-14 mm) and extra-large (15-20 mm) follicles on either of the ovaries were recorded. Number of small follicles (3 to 5 mm), presence of large cysts (more than 20 mm), presence and nature of CL based on the size and blood flow characteristics (Plate 5.a.) were also recorded (Wolfenson *et al.*, 1995; Sartorelli *et al.*, 2005; Ginther, 2016).

3.2.9.2.*Ultrasonographyduring Oestrus*

Animals reported to have oestrus signs were subjected to scanning for confirmation on the day of oestrus(Day 0) and the subsequent day (Day 1), to assess the size of mature follicle, presence of CL and structural features of the reproductive tract.Size and blood flow characteristics of CL and concurrent growth of follicles were also recorded.

3.2.9.3. *Ultrasonography for Detection of Embryonic Death*

Non-return animals were scanned for early indications of pregnancy at 25 days after AI and scanning was repeated at 35 and 45 days of insemination to assess viability and growth of embryo, structural features of the CL and progression of the uterine changes. Features of early pregnancy noticed such as presence of fluid *in utero,* foetal membranes, embryonic structures, blood flow characteristics and foetal heart beat were recorded (Plate 5.b). Number of early pregnancies detected at the three intervals of scanning were compared to detect any embryonic loss happened within 45 days of service.

3.2.10. Blood Sampling

Blood samples were collected by Jugular veni-puncture using syringe and needle. Four millilitersof blood were taken in Vacutainer (5 mL) tubes (CAT- UNIVV CA 4, Vacto Vein clot activator, Canada) and allowed to clot under room temperature (1-2 hours) followed by refrigeration overnight. Serum was separated by centrifugation (4000 x g for 4 minutes) and 1 mL each were transferred into storage tubes of 1.5 ml capacity (Cliklok micro centrifuge tubes, Tarsons products limited, India) and stored in duplicates of one mL aliquots in a deep freezer under -20ºC until estimation.

3.2.10.1. *Stress Associated BiologicalFactors*

Serum samples werethawed and used for the estimation of HSP 70 and cortisol usingELISA Kits. Weekly serum samples of December to May (period of lowest and highest TS) were also used for colorimetric estimation of MDA level to understand the antioxidant protection level.

i. ELISA for HSP 70 in Serum

Serum HSP 70 concentration was estimated by Bovine specific HSP 70 ELISA kits (Chongqing Biospes Co Ltd, China) as per the details given in Appendix 5. In addition to the kit components, microplate washer (Immuno wash - model 1575, Bio Rad laboratories, CA) (Plate 6.a.), micro plate reader (iMark microplate reader - Bio-RAD laboratories) (Plate 6.b.) installed with microplate manager software(Bio Rad Microsoft manager 6.0, Bio Rad Laboratories, CA) and various accessories available at the lab was utilized for ELISA.

HSP 70 values of 544 serum sampleswere estimated,which included samples collected at weekly intervals (Days 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 PP) from all the animals (n=445) as well as 99 post AIsamples collected (3 each) from each of the 33 cows inseminated during the study .

Plate 3.a. LRST model movable trevis used in the study

Plate 3.b. Removable tray of movable trevis for placing the scanner

Plate 4.a. Esoate veterinary ultrasound scanner with SV 3513 probe

Plate 4.b. Ultrasonography of ovary - non echogenic image of the follicle

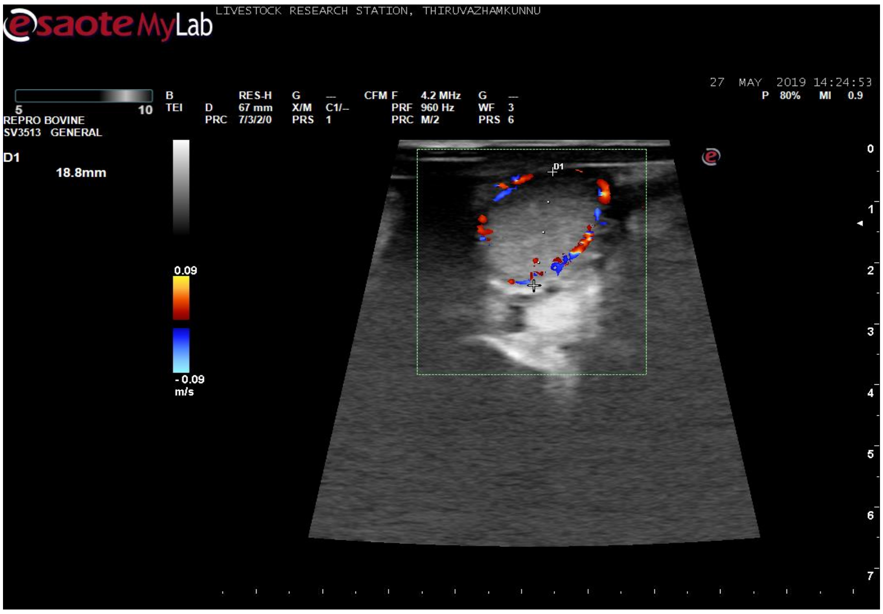

Plate 5.a. Functional corpus luteum with characteristic blood flow

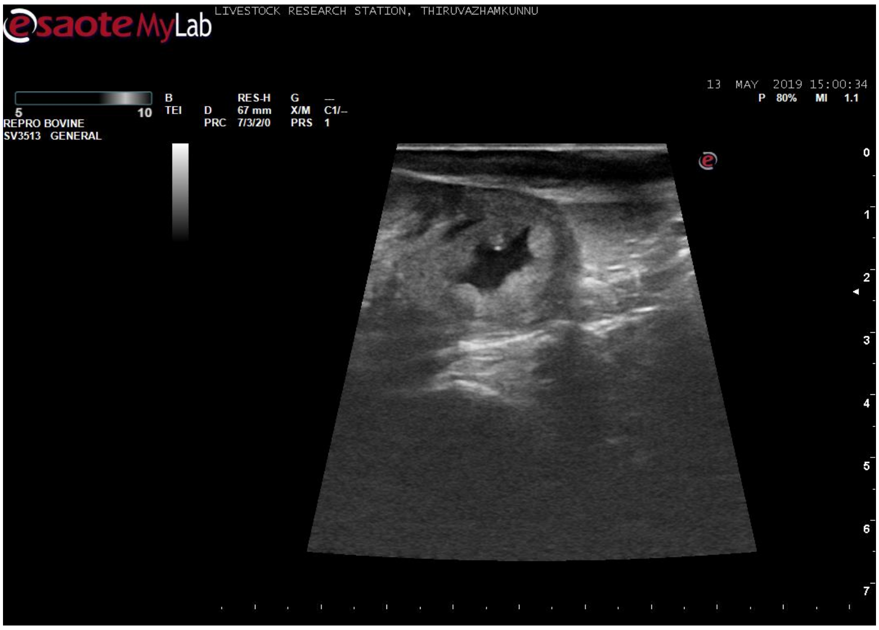

Plate 5.b. Ultrasonographic image at 25th day of pregnancy

Plate 6.a. Bio Rad micro plate washer (Immuno wash Model 1575)

Plate 6.b. Bio Rad ELISA reader (iMark – Canada)

ii. ELISA for Serum Cortisol

Serum cortisol concentration was estimated using ELISA kits (Neogen – USA), which necessitated extraction of cortisol from the serum samples before conducting the assay. Details of the extraction and estimation are described in Appendix 6.All the 445 serum samples collected at weekly intervals were subjected to cortisol assay.

iii. Estimation of Serum Malondialdehyde

MDAwas estimated in the serum samples by Thiobarbituric acid (TBA) colorimetric assay using spectrophotometeras per the details given in Appendix7. Serum samples collected at weekly intervals as mentioned earlier during the period of low (DJF) and high THI (MAM) were subjected to MDA estimation to assess the level of anti-oxidant present in response to TS. Detailed procedure for MDA estimation is given in Appendix 7.

3.2.10.2.Reproductive Hormones

Oestrogen level in the serum samples collected on Day 0 and Day 1 of oestrus (n=68) and P4 levels in serum samples collected routinely at weekly intervals (n=445) and post-service samples (n=99) were analysed using ELISA kits as described below.

i. ELISA for Oestrogen

Serum samples of Day 0 and Day 1 of oestrus were subjected to estimation of E2 using ELISA kit (Calbiotech inc®, El-Cajon Canada) as described in Appendix 8.Sixty eight samples collected from 34 oestrus periods were analysed for oestrogen and the values were compared with TS indicators and other study parameters.

ii. ELISA for Progesterone

Serum samples were subjected to estimation of P4concentration using Pathozyme ELISA Kits (Omega diagnostics® Ltd, U.K.) and the procedure is described in appendix 9. Routine serum samples collected at weekly intervals from day 28 to 91 PP were subjected to P4 estimation. Routine samples were not collected from inseminated animals until return to service or declared negative through ultrasonographic diagnosis of early pregnancy starting from Day 25 post AI. Thus, 445 samples were tested for basal P4 level of early PP period.

Post service serum samples(n = 99)of Day 7, 14 and 21 post AI collected from 33 inseminated animals were tested for post service P4 level using ELISA. Both post partumand post-serviceP4 levels obtained were compared with biological stress indicators as well as climatic factors to find out any relationship between them.

3.2.11. Pregnancy Diagnosis

Besides detection of early pregnancy out of 33 inseminations using USGin Gr. 2, pregnancy diagnosis was performed in all the animals of Gr. 1 and 2 (n=38) at Day 60 and further confirmation at Day 90 post-service by clinico-gynaecological examination.

3.2.11.1. Conception Rate of AI

All the inseminated animals were observed for return to oestrus and non-returns were checked for pregnancy as mentioned before. Proportion of non-returns and conceptions were estimated at each time interval of checking upto 3 months.

3.2.11.2. Pregnancy Loss

Proportion of early pregnancy loss between successive examinations was assessed by comparing the number of conceptions recorded at each time intervals of checking by USG and subsequent gynaecological palpation in Gr. 2, while in Gr. 1 comparison was done between the gynaecological palpation findings at Days 45, 60 and 90 post AI.

3.3. STATISTICAL ANALYSIS

Raw data collected during the study comprised of six years' retrospective period and one year prospective observations was subjected to sorting, grouping and taking the mean values for conducting statistical analysis and interpretation. The whole data were analysed using statistical software SPSS V. 24.0. The data on various parameters were statistically analysed using,mostly one way analysis of variance (ANOVA) followed by multiple comparison tests (MCT) using Duncan MRD and Pearson's correlation methods. However, two way ANOVA with MCT was used for theinteraction of BCS and milk yield across lactation groups and the seasons, Students t test was used for comparingindependent groups such as RR, RT and MDA across two seasons and Chi-square test was used for comparing conception rate across the seasons of the study.The details of the study variables, climatic parameters and their interactions are described.

RESULTS

4. RESULTS

4.1. RETROSPECTIVE STUDY

4.1.1. Weather Parameters in the Past Six Years

Weather parameters collected from weather station recordings over the recent past six years were used for assessing the occurrence of thermal stress (TS).Monthly mean of daily AvT (in ºC) was lowest during January (25.06) and highest in April (28.99). MnT also had the same pattern (18.32 versus 24.16). However, monthly mean of MxT had different pattern with the highest during March (37.04) and the lowest (29.70) was in July. Monthly mean of daily AvRH during the 6 years was highest (97.75 %) in July and lowest (77.37%) in February. The THI calculated from daily AvT and AvRH showed highest monthly mean during April (82.28) as against the lowest of 75.10 during January.

Even though THI 72 is often considered as the demarcation for the zone of thermal comfort for dairy animals, none of the months had THI below 75. Hence comparison of seasonal pattern of variations on the basis of operational classification of the seasons was given more emphasis throughout the studyin order to assess the influence of climatic stress factors on reproductive parameters.

Table 4.1. Maximum, minimum and daily mean values of ambient temperature and relative humidity (Mean ± SE) of the study area during the four seasons

Variable	SON	DJF	MAM	JJA	Mean	F-value
MxT (ºC)	32.17±0.28^{b}	34.01±0.19^{c}	36.00±0.33^{d}	30.08±0.28^{a}	33.06±0.47	84.59
MnT (ºC)	22.79 ±0.28^{b}	19.62±0.31^{a}	23.41± 0.23^{b}	23.19±0.09^{b}	22.25±0.34	52.34
AvT(ºC)	26.15 ±0.08^{a}	25.77 ±0.14^{a}	28.43 ±0.28^{b}	25.62 ±0.14^{a}	26.49 ±0.25	56.35
MxRH (%)	98.03±0.60^{bc}	93.61±1.41^{a}	95.20±1.14^{ab}	99.00±0.24^{c}	96.46±0.64	6.70
MnRH (%)	71.82±3.99^{b}	45.10±1.78^{a}	53.51±3.27^{a}	86.14±2.26^{c}	64.14±3.60	38.87
AvRH (%)	94.54±0.95^{b}	82.61±2.02^{a}	86.98±1.96^{a}	97.32±1.05^{b}	90.36±1.43	18.49

Values with different superscripts in each row varied significantly ($P<0.001$)

Quarterly mean values of major environmental variables recorded during the study period such as maximum, minimum and daily average of AbTand RH are shown in Table 4.1. All of these climatic parameters varied significantly (P<0.001) between the seasons with highest daily mean AbT of 36.00 ºC during MAM and the minimum of 19.62 ºC during DJF.

In climatologic interpretations, maximum, minimum and average temperatures are usually being considered to assess the comfort levels. Like wise,THI values were calculated using daily mean, maximum and minimum valuesof AbT and RH during all the four seasons, to understand the extend of possible variation and are shown in Table 4.2.

Table 4.2.THI values calculated based on the daily average, maximum and minimum recordings of ambient temperature and relative humidity

AbT and RH used for THI	**Seasons**	**THI**		
		Min	**Max**	**Mean**± SE
Mean	SON	77.92	79.15	78.43±0.17^{b}
	DJF	75.87	77.11	76.42± 0.22^{a}
	MAM	80.52	82.51	81.36±0.33^{c}
	JJA	76.86	78.31	77.82±0.21^{b}
Maximum	SON	88.49	91.22	89.55±0.42^{b}
	DJF	91.17	93.06	91.98±0.31^{c}
	MAM	94.02	96.77	95.76±0.49^{d}
	JJA	83.92	87.43	85.99±0.50^{a}
Minimum	SON	69.37	71.60	70.65±0.34^{b}
	DJF	63.56	66.01	64.50±0.36^{a}
	MAM	68.48	71.30	70.02±0.44^{b}
	JJA	71.62	73.05	72.53±0.20^{c}

Values with different superscript varies significantly (P<0.001) between seasons

THI based on daily MxT and MxRH as well as daily MnT and MnRH helped to understand the range of possible variations. While the highest and lowest possible THI values based on daily maximum of AbT and RH ranged from 83.92 (JJA) to 96.77 (MAM) respectively, the lowest THI possible based on daily minimum values of AbT and RH ranged from 63.56 (DJF) and 73.05 (JJA). However, there was an inverse pattern of variation between the AbT and RH so that maximum of these two variables do not occur simultaneously, so that THI based on daily mean values of AbT and RH was more relevant and used for further comparison. Monthly mean THI based on daily AvT and AvRH ranged from 75.87 in DJF to 82.51 during MAM indicative of mild to moderate stress throughout the year.

4.1.2. Productive and Reproductive Status of the Herd during Different Seasons

Animal stock details under different categories of breeding during the study period and its seasonal fluctuations are shown in Table 4.3. The mean figures of cattle in different reproductive phases across the four seasons and the yearly mean of six years are shown. There was no prominent similarity with respect to the pattern of different parameters and the variations were non-significant between seasons indicating more or less similar number of animals under each of the categories across the seasons.

Table 4.3. Mean ± SE of herd performance parameters during different seasons

Cattle in different reproductive phases	**Seasons**				**Mean ± SE of six years**
	SON	**DJF**	**MAM**	**JJA**	
Total number of cattle	92.83 ± 6.32	90.82 ±5.38	90.30 ± 4.29	89.97 ± 6.08	90.98 ± 2.61
Cows in milk	55.06 ± 2.04	56.50 ± 2.68	56.50 ± 3.24	53.61 ± 2.85	55.42 ± 1.30
Breedable cows in milk	18.35 ± 0.67	18.82 ± 0.88	18.82 ± 1.08	17.87 ± 0.94	18.46 ± 0.43
Breedable dry cows	12.60 ± 1.79	11.43 ± 1.48	11.53 ± 1.03	12.55 ± 1.24	12.03 ± 0.67
Breedable heifers	38.90 ± 1.27	39.35 ± 3.07	38.45 ± 3.84	38.72 ± 5.37	38.85 ± 1.97
Total breedable females	69.83 ± 4.28	69.62 ± 1.18	68.80 ± 4.02	69.13 ± 5.73	69.35 ± 2.05
Pregnant cows	33.82 ± 3.30	35.38 ± 3.10	30.30 ± 3.40	28.90 ± 1.66	32.10 ± 1.48
Pregnancy proportion	26.15 ± 2.90	27.57 ± 2.79	23.62 ± 2.50	23.02 ± 2.17	25.09 ± 1.28

Variations between seasons are statistically non-significant ($P>0.05$) for all the parameters

4.1.3. Breeding Activities

Mean ± SE of breeding activities during each of the quarters and the mean of six years are shown in Table 4.4. Mean figures of reproductive parameters such as total number of oestrus detected, number of animals inseminated, number of AI done, proportion of animals inseminated out of those detected in oestrus, number of animals conceived, abortions detected, conception rate of AI, number of calvings among adult females and number of calvings per month are shown. Most of the parameters except conception rate of AI showed lowest mean during MAM, even though variations between seasons were non-significant.

Table 4.4.Annual and seasonal reproductive performance of the herd during the six years

Reproductive parameters	**Seasons**				**Mean ± SE of six years**
	SON	**DJF**	**MAM**	**JJA**	
Oestrus detected	34.6 ± 3.2	33.2 ± 4.3	29.1 ± 3.2	30.0 ± 2.4	31.7 ± 1.6
Animals inseminated	25.8 ± 4.0	29.1 ± 6.1	23.0 ± 1.9	22.7 ± 1.1	25.1 ± 1.9
Total number of AI	25.4 ± 2.8	24.5 ± 4.1	22.9 ± 1.8	23.5 ± 2.5	23.2 ± 1.4
Breeding proportion^{a}	73.4 ± 7.3	73.8 ± 5.1	78.6 ± 8.0	78.3 ± 4.6	76.0 ± 3.1
Animals conceived	7.7 ± 1.2	5.3 ± 0.7	5.3 ± 0.7	6.4 ± 1.0	6.2 ± 0.5
Abortions detected	0.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
Conception rate	37.4 ± 3.1	35.2 ± 7.4	37.5 ± 5.8	36.3 ± 7.6	36.6 ± 2.9
Calvings / adult females	5.6 ± 0.8	3.8 ± 0.5	3.8 ± 0.4	5.1 ± 0.8	4.6 ± 0.3
Calvings per month	7.50 ± 0.99	4.95 ± 0.82	4.90 ± 0.59	6.55 ± 0.84	5.98 ± 0.44

Variations non-significant ($P>0.05$) for all the parameters (a – Number of AI / number of oestrus x 100)

Rather high incidence of prolonged oestrus (signs persisting > 36 hours) was observed in the herd and one of the major management strategies adopted for prolonged oestrus was to repeat the insemination on subsequent days of oestrus prolongation. In this respect, details of oestrus cycles of cows managed with single AI (SAI) or double AI (DAI) (meaning AI done on consecutive days) during the study period are compared between the seasons in Table 4.5.

Table 4.5. Mean ± SE of single and double AI performed during each oestrus period and the conception rate across seasons

Breeding parameters	**Seasons**				**Mean ± SE of six years**
	SON	**DJF**	**MAM**	**JJA**	
Oestrus having AI done	20.7±2.5	19.4±3.8	15.2±1.6	20.4±3.1	18.9±1.4^{ns}
Double AI done per cycle	4.8±0.5	5.7±0.7	4.3±0.8	3.0±0.9	4.3±0.4^{ns}
Single AI done per cycle	15.9±2.2	13.7±3.3	10.9±1.9	17.4±3.8	14.5±1.4^{ns}
DAI out of total AI done (%)	25.7±2.4	32.0±4.6	28.4±6.2	17.9±6.2	26.0±2.6^{ns}
Single AI conception rate (%)	30.4±4.9	39.1±10.3	34.5±7.5	32.2±8.8	34.0±3.9^{ns}
DAI Conception rate (%)	51.0±3.2^{b}	47.1±3.4^{b}	40.9±3.2^{ab}	31.5±6.9^{a}	42.6±2.6*

*. Significant ($P<0.05$), Means with different superscript varies significantly between seasons, ns- ($P>0.05$)

Number of oestrus cycles where in breeding was done, total number of AI done, breeding by single and double AI during the cycle, proportion of DAI out of total cycles of breeding and conception rates of single AI and DAI are shown for the seasons along with the yearly mean figure. The pattern of variation between seasons was different for all the parameters and non-significant except for DAI conception. Conception rate of DAI was better than that of single AI during the study years (42.6 versus 34.0) and the variation was significant across seasons (P<0.05) with the highest and lowest conception rates during SON and JJA respectively.

4.1.4. Fertility Indices

Season wise summary of fertility indices of the herd during the study period are given in Table 4.6. There was no striking similarity between the patterns of variation of these parameters between seasons and the variations were non-significant between seasons.

Table 4.6.Mean ± SE of herd fertility indices of cows during the study period

Fertility indices	Seasons				Mean ± SE of six years
	SON	DJF	MAM	JJA	
Age at first calving (months)	31.5±6.4	35.3±1.4	35.3±0.6	36.0±0.6	34.5±1.6
Onset of postpartum oestrus (days)	77.8±13.5	90.0±15.3	57.5±6.5	62.0±3.9	71.8±5.7
Service period (days)	120.2±19.6	126.8±17.2	103.5±17.1	93.0±10.1	110.9±8.1
Inter-oestrus interval (days)	38.2±2.3	39.4±2.1	40.5±3.4	39.5±1.8	39.4±1.2
AI per conception	2.6±0.2	2.9±0.4	2.8±0.3	2.5±0.2	2.7±0.1
Calving-conception interval (days)	223.2±27.8	251.2±33.3	194.5±38.4	229.8±34.3	224.7±16.2
Inter calving interval (days)	467.7±23.9	526.3±38.7	469.5±42.6	455.7±29.6	479.8±17.1

Variations between seasons are statistically non-significant (P>0.05) for all the parameters

4.1.5. Seasonality of Fertility Parameters

Comparison of the seasonal averages showed no significant correlation between fertility parameters and weather parameters such as AbT or THI. However, RH showed mild to moderate correlation with some of the fertility parameters as shown in Table 4.7. While the number of single AI done per cycle showed highly significant (P<0.01) positive correlation with daily MxRH, all other correlations were significant at 5 per cent level. Further the correlation of RH with pregnant cow proportion of the herd, proportion of double AI out of total AI done and single AI conception rate were negative, while all other correlations were positive, even though correlation of these parameters were not uniform across different levels of RH.

Table 4.7. Correlation (p-value) of fertility parameters with RH across seasons

Parameter	**Correlation coefficient and significance of RH**		
	MnRH	**MxRH**	**AvRH**
Pregnant cow proportion		-0.456 (0.025) *	-0.407 (0.048) *
Number of calving	0.405 (0.049)*		
Calving per adult female	0.476 (0.019) *		
Total number of AI		0.425 (0.039) *	
Oestous cycles having breeding done		0.503 (0.012) *	0.419 (0.041) *
Proportion of oestrus inseminated		0.486 (0.016) *	
Total number of conception	0.409 (0.047) *		
Conception rate of AI	0.437 (0.033) *		
Double AI among total AI (%)		-0.508 (0.011) *	-0.448 (0.028) *
Single AI done per cycle		0.570 (0.004)**	0.503 (0.012) *
Conception from single AI	0.416 (0.043) *		
Single AI conception rate		-0.474 (0.019) *	

* significant ($P<0.05$), ** significant ($P<0.01$).

Across seasons, there were highly significant correlations between different breeding and fertility parameters. Some of the parameters having highly significant negative correlation are shown in Table 4.8.

These correlations imply that as the conception rate decreases total number of AI during the season increased. Similarly, as the proportion of double AI in the same oestrus increases, total number of oestrous cycles with breeding decreased. Increase in the number of single AI was resulted from reduced conception so that proportion of pregnant animals in the herd decreased.

Also age at first calving of heifers decreased as the conception rate of AI increased. Increase of service period and inter calving interval were associated with decrease in the number of calvings. Further increase of AI required per calving was found to decrease pregnancy proportion and total number of pregnant cows in the herd.

Table 4.8. Correlation between fertility parameters across seasons

	Parameter	Dependant variable	Corr. coeff. (p-value)
1	Total number of AI	Conception rate of total AI	-0.570**(0.004)
		Conception rate of single AI	-0.536** (0.007)
2	Double AI out of total AI (%)	Oestrous cycles inseminated	-0.536 ** (0.007)
3	Number of single AI	Proportion of pregnant animals	-0.508** (0.001)
4	Conception rate of AI	Age at first calving	-0.651**(0.001)
5	Number of calving	Service period	-0.481** (0.017)
6		Inter calving interval	-0.522** (0.009)
7	AI per calving	Pregnancy proportion in the herd	-0.560** (0.004)
		Number of pregnant cows	-0.582** (0.003)

** significant (P<0.01)

Year wise variations of the breeding and fertility parameters during the study period are shown in Table 4.9 and the details are also shown in appendix 1.

Table 4.9. Stock and fertility parameters of significant variation between years

Animal stock details	F-value	p-value	Fertilityparameters	F-value	p-value
Total adult females	3.92	0.014*	Oestrus with AI done (%)	10.98	<0.001**
Total cows in the herd	9.99	< 0. 001**	Double AI done	3.56	0.020*
Milking cows in the herd	6.14	0.002**	Double AI proportion (%)	4.94	0.005**
Dry cows in the herd	10.55	< 0. 001**	DAI conception	3.12	0.033*
Number of pregnant cows	4.86	0.005**	Single AI during oestrus	2.78	0.049*
Herd preg. proportion	8.15	< 0. 001**	Single A I conception	2.80	0.048*
Breedable dry cows	7.57	<0.001**	Total conception	3.31	0.027*
Breedable milking cows	6.08	0.002**	AI Per conception	7.48	<0.001**
Breedable heifers	11.45	< 0. 001**	Conception rate (%)	5.26	0.004**
Total breedable females	5.17	0.004**	Inter oestrus interval	2.97	0.040*

* significant (P<0.05), ** significant (P<0.01).

Highly significant variation of the stock related parameters between the years of study as shown in the table might be due to management differences. Even though various fertility parameters showed significant variation between the years, there was no unique pattern across these years. To illustrate, conception rate of AI was more or less similar during 2013-14 (25.36 %) and 2014-15 (24.50 %), highest during 2018-19 (56.14 %) and intermediate for other three years such as 2015-16 (32.63 %), 2016-17 (41.11 %) and 2017-18 (39.85%) and the variation between these years were highly significant ($P<0.001$). Whereas number of AI done was lowest to highest such as 19.33, 19.91, 22.58, 23.50, 31.33 and 34.03, respectively for the years in the order of 2013-14, 2018-19, 2017-18, 2016-17, 2014-15 and 2015-16.

Since lot of management factors might have influenced these parameters between the years, usefulness of these parameters are very limited to study the influence of TS, especially since weather parameters did not vary significantly between the years.

Comparison of the study parameters between the periods of low and high THI (72 to 78 and more than 78) did not show significant variation. However, time series analysis showed significant correlation of weather parameters with breeding parameters as well as between different reproductive parameters as shown in Table 4.10 and 4.11.

Table 4.10.Correlation of breeding parameters with weather parameters across six years

Parameters	**Weather parameters**						
	MxT	**MnT**	**MxRH**	**MnRH**	**AvT**	**AvRH**	**THI value**
Cows in milk	0.229	0.064	-0.053	-0.301*	0.182	-0.069	0.164
Breedable females	0.046	0.081	-0.201	0.012	0.035	-0.175	-0.046
Total adult females	0.026	0.156	-0.031	-0.099	-0.019	-0.034	-0.041
Total number of calving	-0.154	0.292*	0.012	0.227	0.021	0.099	0.068
Calvings per adult females	-0.214	0.193	0.072	0.281*	-0.075	0.182	0.002
Oestrus detected	-0.129	-0.189	0.056	0.131	-0.210	0.079	-0.191
AI done	-0.039	-0.258*	0.228	-0.055	-0.168	0.137	-0.117
Oestrus cycles inseminated	-0.262*	-0.047	0.389**	0.135	-0.291*	0.331**	-0.173
Double AI done	0.020	-0.217	-0.191	-0.027	-0.085	-0.134	-0.142
Total number of AI	-0.237*	-0.110	0.303**	0.117	-0.295*	0.266*	-0.204
Breedable females inseminated	-0.149	0.087	0.346**	-0.033	-0.146	0.258*	-0.046
Total number of conception	-0.235*	0.035	0.125	0.357**	-0.131	0.172	-0.056
Conception rate	0.002	-.023	-0.177	0.127	0.057	-0.137	0.012

(Mx-Maximum, Mn -minimum, Av-Average, T-Temperature, RH-Relative Humidity)

*significant ($P<0.05$), ** Significant ($P<0.01$)

Table 4.11. Correlation (Pearson's coefficient) between reproductive performance parameters

Parameters	Cows in milk	Breedable Females	Adult Females	Number of Calving	Calvings per adult females	Number of Oestrus	Number of single AI done	Oestrous cycles of AI done	DAI done	Total number of AI	% of oestrus with AI	Number of conception
Breedable Females	0.316**											
Adult Females	0.549**	0.841**										
Number ofCalving	0.128	0.257*	0.280*									
Calvings per adult Females	0.000	0.089	0.045	0.818**								
Number of oestrus	-0.172	0.084	-0.045	-0.274*	-0.216							
Number of AI done	0.123	-0.046	-0.035	-0.389**	-0.296*	0.656**						
Oestrous cycles of AI done	0.152	-0.071	0.095	-0.290*	-0.242*	0.585**	0.724**					
Double AI done	-0.273*	0.020	-0.142	-0.143	-0.139	0.565**	0.447**	0.083				
Total number of AI	0.058	-0.060	0.045	-0.312**	-0.267*	0.715**	0.807**	0.953**	0.382**			
% of oestrus with AI	0.231	-0.218	0.098	-0.053	-0.065	-0.315**	0.197	0.494**	-0.193	0.400**		
Number of conception	0.020	0.153	-0.040	-0.032	-0.027	0.362**	0.269*	0.217	0.304**	0.294*	-0.063	
Conception rate	0.017	0.190	-0.037	0.267*	0.208	-0.172	-0.311**	-0.495**	0.081	-0.435**	-0.367**	0.623**

*Significant (P<0.05) ; ** Significant (P<0.01)

4.2. PROSPECTIVE STUDY

Carried out for a period of 12 months from September 2018 to August 2019 and prospective data collected include:

4.2.1. Weather Parameters

Daily recordings of AbTand RH were collected from macro and micro climatic recording devices used during the study. Monthly averages and the differences between macro and micro recordings of these two variables are shown in Table 4.12.

Table 4.12. Monthly averages of ambient temperature and relative humidity from macro and micro climatic recordings and their differences

Month and year	Macro climate		Micro climate		Difference	
	AbT (ºC)	RH (%)	AbT (ºC)	RH (%)	AbT (ºC)	RH (%)
Sep 2018	26.49	90.45	28.35	83.84	1.87	-6.61
Oct 2018	25.97	93.96	28.64	79.08	2.67	-14.88
Nov 2018	26.51	88.45	29.00	73.78	2.49	-14.68
Dec 2018	27.06	90.05	28.55	72.39	1.49	-17.66
Jan 2019	24.36	75.45	26.78	62.85	2.43	-12.60
Feb 2019	27.62	75.63	29.89	63.43	2.27	-12.20
Mar 2019	28.82	80.11	31.48	64.45	2.66	-15.66
Apr 2019	29.41	84.32	32.02	69.22	2.61	-15.10
May 2019	29.40	85.39	29.86	79.86	0.46	-5.53
Jun 2019	27.22	92.97	29.12	81.69	1.90	-11.28
Jul 2019	25.58	95.60	27.35	86.18	1.78	-9.42
Aug 2019	25.10	97.35	26.89	88.02	1.79	-9.33
Mean	26.96**	87.48**	29.00**	75.40**	2.03**	-12.08**
F- Value	100.77	68.16	64.86	92.10	10.30	13.38
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

** Significant (P<0.001)

The months of highest AvT (ºC)were different for macro and micro recordings being May (29.40) and April (32.02), respectively even though the lowest was during January for both. However, highest monthly AvRH (%) was during August from both macro (97.35) and micro (88.02) recordings and the lowest was (75.45 and 62.85, respectively) during January.

Between months there were highly significant variations for AbT and RH obtained from macro and micro recordings as well as their differences between macro and micro recordings. AbT was more inside the shed than outside with overall mean difference of 2.03ºC, whereas RH was found to be more outside with a mean difference of 12.08 %. Higher AbT and low RH inside the shed than outside and *vice versa* formed an indication of the inverse relationship between the AbT and RH.

4.2.1.1. Temperature Humidity Index

Quarterly averages of THI values calculated based on daily AvT and AvRH from micro and macro climatic recordingsare shown in Table 4.13.

Table 4.13. Mean values of ambient temperature, relative humidity and THI of four seasons from macro and micro climatic recordings

Category	Weather parameters	Seasons				F value	p value
		SON	DJF	MAM	JJA		
Macro climate	AvT(ºC)	26.32^{b}	26.34^{b}	29.21^{a}	25.96^{b}	6.093*	0.018
	AvRH (%)	90.95^{bc}	80.37^{a}	83.27^{ab}	95.30^{c}	6.243*	0.017
	THI	78.31	77.14	82.14	78.18	4.011^{ns}	0.520
Micro climate	AvT (ºC)	28.66^{b}	28.40^{b}	31.12^{a}	27.78^{b}	4.970*	0.031
	AvRH (%)	78.90^{bc}	66.22^{a}	71.17^{ab}	85.29^{c}	6.694*	0.014
	THI	80.61^{ab}	78.47^{a}	83.20^{b}	80.05^{a}	5.091*	0.029

AvT – Daily average temperature, AvRH – Daily average relativehumidity, ^{ns} non-significant (P>0.05)

* Significant(P<0.05), Means with differentsuperscripts varysignificantly between columns

Seasonal pattern of AvT, AvRH and THI were similar for macro and micro climatic recordings. Highest quarterly means of AbT and THI were during MAM. Lowest of AbT and THI were during JJA and DJF, respectively;while the highest and lowest of RH were during JJA and DJF, respectively. Thus besides the coincidence of high AbT and THI during MAM, lowest RH and THI were during DJF. Further, JJA formed the season of highest RH and lowest AbT, while SON maintained the moderate level with respect to all the three weather parameters.

Even though THI is a composite index arrived from daily AvT and AvRH, the correlation between THI values and other weather parameters such as MxT, MnT, MxRH and MnRH are shown in Table 4.14. Correlation of AvRH with THI was less compared to that of AvT indicating relative contribution of these two variables into the THI. Other than AvT and AvRH, only MnT showed highly significant positive correlation with THI and there was no significant correlation of THI with MxT, MxRH and MnRH.

Table 4.14. Correlation of Temperature HumidityIndex with other weather parameters

Parameter	Correlation coefficient ofweather parameters					
	AvT	AvRH	MnT	MxT	MnRH	MxRH
THI	0.930**	0.412*	0.894**	0.432	0.080	-0.191
(p-value)	(<0.001)	(0.015)	(<0.001)	(0.161)	(0.804)	(0.552)

* Significant (P<0.05), ** Significant (P<0.01)

AbT, RH and THI values based on macro and micro climatic recordings and their differences are compared between the half years in Table 4.15. Both micro and macro climatic recordings of RH showed highly (P<0.01) significant variation between the half years of rainy and non-rainy seasons, while the variation was not significant for temperature and THI. At the same time, none of these weather parameters showed significant variation between long day and short day half years.

Table 4.15. Half yearly averages of ambient temperature, relative humidity and THI based on macro climatic and micro climatic recordings and their differences

Period (Months)	Macro climate			Micro climate			Difference		
	AbT (ºC)	RH (%)	THI	AbT (ºC)	RH (%)	THI	AbT (ºC)	RH (%)	THI
Short days (Sep to Feb)	26.34	85.67	77.72	28.54	72.56	79.54	2.20	-13.10	1.82
Long days (Mar to Aug)	27.59	89.29	80.27	29.45	78.24	81.78	1.87	-11.05	1.52
Rainy season (Jun to Nov)	26.14	93.13	78.26	28.23	82.10	80.37	2.08	-11.03	2.10
Non-rainy(Dec to May)	27.78	81.82	79.60	29.77	68.70	80.83	1.99	-13.12	1.23

As shown in Table 4.14, THI value from macro weather parameters did not vary significantly between seasons (F value = 4.011, p value = 0.052), while micro climatic THI showed significant variation (F value = 5.091, p value = 0.029). Since micro climate is the one directly affecting the animal and varied significantly, THI value from micro climate was used for subsequent analysis.

4.2.1.2. Other Climatic Stress Factors

Monthly averages of MxT, MnT, MxRH and MnRH recorded within the animal sheds are shown in Table 4.16. The extent of daily variation of these variables determines the severity of TS exposed. Hence, the variation between maximum and minimum of AbT and RH in terms of actual difference and as percentof the minimum recorded value are also included in Table 4.16.

Table 4.16. Monthly averages of maximum and minimum of temperature and relative humidity and their daily variations in actual terms and as per cent of daily minimum value

Months	Temperature		Relative Humidity		Daily variation - AbT		Daily variation - RH	
	Min ºC	Max. ºC	Min. %	Max. %	ActualºC	%	Actual %	%
Sep	24.91	31.80	72.77	94.91	6.89	27.64	22.14	30.42
Oct	24.04	33.25	62.85	95.32	9.21	38.29	32.47	51.66
Nov	23.79	34.21	53.91	93.65	10.43	43.84	39.74	73.72
Dec	23.22	33.89	51.74	93.04	10.66	45.92	41.31	79.84
Jan	19.72	33.85	35.74	89.97	14.12	71.60	54.23	151.76
Feb	23.35	36.44	37.18	89.68	13.09	56.06	52.50	141.22
Mar	25.19	37.78	39.48	89.42	12.59	49.98	49.93	126.46
Apr	26.27	37.77	47.50	90.94	11.50	43.79	43.44	91.45
May	27.47	32.26	70.91	88.81	4.79	17.45	17.90	25.24
Jun	25.51	32.73	68.98	94.39	7.22	28.29	25.42	36.85
Jul	24.49	30.21	77.47	94.88	5.72	23.35	17.41	22.48
Aug	24.27	29.52	80.56	95.49	5.25	21.62	14.92	18.53
Mean ± SE	24.35 ± 0.55	33.64 ± 0.77	58.26 ± 4.65	92.54 ± 0.75	9.29 ± 0.94	38.99 ± 4.63	34.28 ± 4.17	70.80 ± 13.88

Lowest means of MnT and MxT was in January and August, respectively, while the corresponding highest means were attained during May and March. Similarly, lowest means of MnRH and MxRH was in January and May respectively and highest of both were in

August.The difference between MnT and MxT was increasing from September to January, thereafter declined more or less continuously across the months until reaching the minimum difference in May and showed an increase again in the subsequent months. The difference between MnRH and MxRH also showed consistent increase from September to January and declined thereafter to the lowest in August.

Quarterly averages of maximum and minimum of AbTand RHand extent of variations are shown in Table 4.17.While MnT and MxT attained highest during MAM, lowest of these two variables were during DJF and JJA, respectively. Similarly, highest means of MnRH and MxRH were during JJA, while the lowest were during DJF and MAM, respectively. The differences between MnT and MxT had similar seasonal pattern as that of MnRH and MxRH with the lowest and highest variations during JJA and DJF, respectively; also showing inverse relationship with day length pattern. However, the variations of MxT and the difference between MnT and MxT were not significant between seasons indicating persistence of high AbT across the seasons.

Table 4.17. Quarterly mean ± SE of maximum and minimum temperature and relative humidity and the extent of variations between seasons

Weather parameters	**Season**					**F value**	**p value**
	SON	**DJF**	**MAM**	**JJA**	**Total**		
Min. AbT (°C)	24.25 ± 0.34^{ab}	22.09 ± 1.19^{a}	26.30 ± 0.66^{b}	24.75 ± 0.38^{b}	24.35 ± 0.55	5.754*	0.021
Max. AbT (°C)	33.08 ± 0.70	34.72 ± 0.86	35.93 ± 1.84	30.82 ± 0.98	33.64 ± 0.77	3.527^{ns}	0.068
Min RH (%)	63.17 ± 5.45^{bc}	41.55 ± 5.11^{a}	52.63 ± 9.43^{ab}	75.67 ± 3.46^{c}	58.25 ± 4.65	5.431*	0.025
Max RH (%)	94.62 ± 0.50^{b}	90.89 ± 1.08^{a}	89.72 ± 0.63^{a}	94.92 ± 0.32^{b}	92.54 ± 0.75	14.414**	0.001
Max-Min AbT	8.84 ± 1.04	12.62 ± 1.03	9.62 ± 2.44	6.06 ± 0.59	9.28 ± 0.94	3.459^{ns}	0.071
Max- Min RH	31.45 ± 5.11^{ab}	49.34 ± 4.05^{b}	37.09 ± 9.78^{ab}	19.25 ± 3.17^{a}	34.28 ± 4.17	4.221*	0.046

* Significant(P<0.05), ** Significant (P<0.01), ^{ns} Non-significant
Means with different superscripts vary significantly within a column

4.2.2. Seasonal Pattern of Thermal Stress

All the major climatic stress factors varied considerably between seasons. Since interaction between different weather parameters involved synergistic or antagonistic effects, the combined influence of various climatic factors in producing TS was assessed by comparing the seasonal pattern of weather parameters as well as the study paramaeters, in addition to the correlation of individual weather parameters with the study parameters.

4.2.3. Influence of Thermal Stress on Animals

The influence of TS on animal system was assessed based on the changes taking place in response to TS exposure. Various general factors taken into consideration included:

Physical - Changes in feed intake, milk production and BCS
Physiological - Changes in respiratory rate and rectal temperature
Biological - Changes in the serum levels of HSP 70, Cortisol and MDA

In addition to these general factors, fertility related parameters studied were

Endocrinological - Serum levels of oestrogen and progesterone
Ovarian changes - Follicular growth pattern and presence of luteal structures
Behavioural - Occurrence and intensity of oestrus
Conception - Occurrence, successful maintenance and interim loss of conception

Variations of these parameters were compared with weather parameters and TS indicators to find out any possible association between them over time.

4.2.3.1. Stress Indicators

Among the biological factors mentioned, HSP 70, Cortisol and MDA were estimated in the serum samples and the details are given under sub-section4.2.10.1. The levels of these stress indicators and weather parameters were compared with all other study parameters to confirm the influence of TS. As the initial step to find out the occurrence of physiological stress in response to seasons, climatic stress factors were compared with biological stress indictors such as HSP 70 and cortisol levels in serum. Quarterly averages of HSP 70 and cortisol levels in serum are shown in Table 4.18.

Table 4.18.Mean ± SE of HSP 70 and cortisol levels in the serum across seasons.

Variable	**Season**				**F-value**	**p-value**
	SON	**DJF**	**MAM**	**JJA**		
HSP 70 (ng/mL)	2.09 ± 0.20^{a}	2.63 ± 0.07^{ab}	6.24 ± 0.51^{c}	2.98 ± 0.13^{b}	50.95**	< 0.001
Cortisol (ng/mL)	8.84 ± 0.30^{bc}	6.84 ± 0.61^{a}	9.44 ± 0.25^{c}	7.80 ± 0.33^{ab}	8.762**	< 0.001

** Significant (P<0.001), Values with different superscripts vary significantly between columns

HSP 70 values varied significantly between seasons (P<0.001), with the highest in MAM and lowest during SON. The level during MAM (6.24 ng/mL) was almost three folds compared to other three seasons even though the variation among these three seasons were also significant. Cortisol levels also varied significantly between seasons with the highest during MAM (9.44 ± 0.25 ng/mL). However the lowest cortisol level (6.84 ± 0.61 ng/mL) during DJF varied significantly from all other seasons except that of JJA.

Correlation of HSP 70 and cortisol with weather parameters are shown in Table 4.19. HSP 70 level was found to be significantly influenced by AvT (P<0.01) and THI (P<0.05) and negatively influenced (P<0.05) by MnT and MxRH. At the same time cortisol level was not influenced significantly by any of the weather parameters studied again indicating other regulatory factors for the level of cortisol

Table 4.19. Correlation of HSP70 and cortisol with weather parameters

Stress indicators	**Weather parameters**						
	THI	**AvT**	**AvRH**	**MxT**	**MnT**	**MxRH**	**MnRH**
HSP 70 (p-value)	0.701* (0.001)	0.752** (0.001)	-0.256 (0.422)	0.494 (0.102)	-0.619* (0.032)	-.619* (0.032)	-0.187 (0.562)
Cortisol (p-value)	0.314 (0.320)	0.358 (0.254)	-0.159 (0.623)	0.313 (0.323)	-0.186 (0.562)	-0.186 (0.562)	-0.147 (0.648)

*. Significant (P<0.05), **. Significant (P<0.01)

4.2.4. Animals Selected for the Study

During 12 months of the study, a total of 82 PP cows (Gr. 1 plus 2) were observed for general physical and reproductive parameters, which consisted of 22 cows eachunder the study every month,belonging to their Day 7 to 136 days PP. Parity of these cows ranged from 2 to 5 with replications for each parity being 28, 18, 23 and 13, respectively. Out of the 82, 60 cows were included for detailed investigation (Gr. 2) as well between days 28 to 91 PP and remaining 22 cows (Gr. 1) were observed only for generalparameters.

4.2.5. Routine Management

Animals under the study were confined to one half of the milking barn without major disturbances and ensuring uniform management. Feeding, cleaning, milking and other general healthcare measures wereprovided uniformly complying with standard practices as mentioned earlier

4.2.6. General Parameters

Details of daily feeding with fodder and concentrate, and milk yield were regularly recorded and body condition scoring of the animals was performed at weekly intervals.

4.2.6.1. Effect of TS on Dry Matter Intake

Comparison of dry matter intakeby the study animals during the four seasons are shown in Table 4.20. Mean daily intake in Kg was highest (13.46) during MAM and the lowest during SON (11.71) and the intake was more or less same during DJF and JJA. DMIof MAM was found to be significantly high ($P<0.05$) compared to other seasons.

Table 4.20. Comparison of dry matter intake (in Kg) between four seasons of the study

Season	Dry matter intake (Kg)		
	Minimum	Maximum	Mean + SE
SON	11.66	11.78	11.71 ± 0.03^{a}
DJF	11.54	13.54	12.59 ± 0.57^{ab}
MAM	13.16	13.63	13.46 ± 0.15^{ab}
JJA	12.08	12.08	12.66 ± 0.31^{b}
Overall	11.57	11.54	12.60 ± 0.23*
F – value=4.484, p- value = 0.040			

*. Significant ($P<0.05$); Meanshaving different superscripts vary significantly

Correlation of DMI with physical parameters such as milk yield and BCS,and weather parametersare shown in Table 4.21. Even though BCS was found to have significant positive correlation ($P<0.05$), none of the weather parametersor milk yield was found to have significant association with dry matter intake.

Table 4.21. Correlation of feed intake with BCS, milk yield and weather parameters

Study Parameter	Variables		Weather parameters				
	BCS	Milk yield	MnT	MxT	MnRH	MxRH	THI
DMI (p-value)	0.697* (0.012)	0.322 (0.308)	0.162 (0.614)	0.326 (0.302)	-0.302 (0.340)	-0.719** (0.008)	0.198 (0.536)

*. Significant ($P<0.05$)

4.2.6.2. Influence of TS on Milk Yield

Comparisons of average milk yield of the cows categorized on the basis of days of lactation and across four seasons are shown in Table 4.22.

Table 4.22.Daily milk yield (litres) at four stages of lactation across the four seasons

Lactation days	**Average milk yield (L)**				
	SON	**DJF**	**MAM**	**JJA**	**Overall**
6 -30	12.63 ±1.29 (n=16)	11.41 ± 0.49 (n=19)	11.61 ± 0.53 (n=22)	10.75 ± 0.92 (n=20)	11.60 ± 0.42^{a} (n=77)
31-60	10.46 ±1.10 (n=15)	10.68 ± 0.31 (n=22)	11.03 ± 0.06 (n=18)	10.57 ± 0.24 (n=14)	10.65 ± 0.28^{a} (n=69)
61-90	8.55 ± .92 (n=13)	8.12 ± 0.14 (n=20)	10.12 ± 0.12 (n=19)	9.34 ± 0.62 (n=17)	8.93 ± 0.52^{b} (n=69)
91 and above	8.54 ± 0.36 (n=14)	8.74 ± 0.12 (n=5)	10.31 ± 1.18 (n=7)	8.79 ± 0.80 (n=15)	9.06 ± 0.39^{b} (n=41)
Overall	10.18 ± 0.80 (n=58)	9.83 ± 0.46 (n=66)	10.86 ± 0.33 (n=66)	9.86 ± 0.39 (n=66)	10.14 ± 0.27 (n=256)

F-value for between lactation stages = 7.967** ; p-value = 0.001

F-value for between Season= 0.923^{ns}; p-value = 0.443

F-value for interaction = 0.545^{ns}; p-value = 0.829

** significant (P<0.01); Means having different superscriptsvary significantly

Irrespective of the season, milk yield was significantly high during the first two stages of lactation (upto 2 months postpartum). However, between seasons there was no significant difference. Overall milk yield was comparatively better during MAM and the obvious reason appeared to be increased dry matter intake and hike in milk production of all lactation stages during the season. Milk production declined rapidly from the third month, whereas those animals attained 61-90 days and above 90 days of lactation during MAM suffered little reduction of yield compared to others.

4.2.6.3. Influence of TS on Body Condition Score

Monthly averages of BCS are compared between seasons in Table 4.23. The BCS was significantly low during SON compared to other three seasons having almost same BCS and is comparable with the low dry matter intake during the season.

Table 4.23. Comparison of mean body condition scores between seasons

Season	Mean BCS	Std. Error
SON	2.87^{b}	0.01
DJF	3.07^{a}	0.06
MAM	3.17^{a}	0.01
JJA	3.05^{a}	0.04
Overall	3.03**	0.03
F-value = 7.724; p-value = 0.010		

Since increased milk production leads to loss of body condition, BCS was sorted based on the stage of lactation and is compared betweenthe four seasons in table 4.24.While actual BCS was significantly low during SON, BCS adjusted for lactation influence does not vary much between seasons. This implies that whatever difference in BCS noticed between seasons was due to the effect of lactation, even though the variation of BCS between lactation groups was also not significant (F-value=1.692 and p-value = 0.188).

Correlations of BCS and milk yield before and after adjusting for the stage of lactation with weather parameters are shown in Table 4.25. There was significant (P<0.01) negative correlation between lactation stage adjusted milk yield and MxRH and significant positive correlation of BCS adjusted for lactation with MxT. Dry matter intake was found to have significant positive correlation (0.697, p-value 0.012) with BCS as already shown in Table 4.21.

Table4.24. Body condition score across four periods of lactation and four seasons

Days of lactation	BCS during season				
	SON	DJF	MAM	JJA	Mean
6-30	3.05 ±0.06	2.93 ± 0.06	3.00 ± 0.07	2.99 ± 0.02	2.99 ± 0.03
31-60	2.97 ±0.09	2.98 ± 0.06	3.00 ± 0.09	2.99 ± 0.05	2.98 ± 0.03
61-90	3.10 ± 0.07	3.10 ± 0.14	3.05 ± 0.06	3.03 ± 0.08	3.08 ± 0.04
Above 90	3.18 ± 0.05	3.00 ± 0.06	3.20 ± 0.07	3.13 ± 0.03	3.13 ± 0.03
Overall	3.10± 0.00	3.00 ± 0.07	3.10 ± 0.04	3.05 ± 0.03	3.06 ± 0.02
F-value for between lactation stages = 1.692^{ns}; p-value = 0.188 F-value for between Season= 0.408^{ns}; p-value = 0.748 F-value for interaction = 0.924^{ns}; p-value = 0.518					

Table 4.25.Correlation of the mean values of BCS and milk yield (actual and lactation stage adjusted values) with weather parameters

Parameter	Weather parameters						
	MxT	MnT	MxRH	MnRH	AbT	RH	THI
BCS (actual) (p-value)	0.407 (0.190)	0.263 (0.409)	0.207 (0.519)	-0.446 (0.147)	0.481 (0.113)	-0.378 (0.226)	0.321 (0.309)
BCS (adjusted) (p-value)	0.661* (0.019)	0.477 (0.117)	0.183 (0.570)	-0.465 (0.128)	-0.108 (0.465)	0.113 (0.446)	-0.049 (0.743)
Milk yield (actual) (p-value)	0.293 (0.356)	0.115 (0.722)	0.039 (0.903)	-0.250 (0.434)	0.303 (0.338)	-0.249 (0.435)	0.222 (0.488)
Milk Yield (adjusted) (p-value)	0.092 (0.788)	0.311 (0.352)	-0.781** (0.005)	0.319 (0.339)	0.185 (0.236)	-0.062 (0.695)	0.176 (0.259)

*. Significant (P<0.05); **.Significant (P<0.01)

Among the general physical parameters, level of feeding had significant positive correlation (P<0.05) with HSP 70 as well as cortisol and BCS was influenced (P<0.05) by HSP 70 alone as shown in Table 4.26. While there was no significant correlation of milk yield with HSP 70 or cortisol

Table 4.26. Correlation of HSP 70 and cortisol with DM intake, milk yield and BCS

Parameter	DMI	Milk yield	BCS
HSP 70 (p-value)	0.707* (0.01)	0.252 (0.43)	0.686* (0.014)
Cortisol (p-value)	0.589* (0.04)	0.538 (0.071)	0.115 (0.723)

*. Significant (P<0.05)

4.2.7. Reproductive Management

Oestrus detected by visual observation at 6-8 hour interval was confirmed through clinico-gynaecological examination only during day timeand AI in required cases was performed without further delay.

4.2.7.1. Oestrus Detection Rate (ODR)

Number of oestrus detected and animals involved during the four seasons are shown in Table 4.27. During the study period of one year, a total of 82 cows were observed for oestrus between second to fourth months PP. Accordingly, during each of the calendar months, there were at least 22 cows under the observation. A total of 63 oestrus periods were detected in 54 animals, which included 9 animals exhibited oestrus more than once, while 34 cows remained anoestrus. Cows under the detailed study(Gr. 2) showed 52 (82.54 %) oestrus and only 11 (17.46 %) oestrus were detected among the remaining cows under general observation alone (Gr. 1). The ODR showed highly significant(chi square p-value = 0.002) variation between seasons with the highest during DJF and lowest in JJA.

Table 4.27.Season wise distribution of oestrus detected, animals observed and proportion of oestrus among the observed animals

Season	Number of oestrus detected			Animals observed	Animals detected in oestrus	ODR (%)
	Gr. 1	Gr. 2	Total			
SON	2	11	13	22	12	54.55^{b}
DJF	4	23	27	22	20	90.91^{a}
MAM	4	11	15	22	14	63.64^{b}
JJA	1	7	8	22	8	36.36^{b}
Overall	11	52	63	82^{a}	54	71.59**

Chi square = 14.379; **. p-value = 0.002 a- Total cows under study

4.2.7.2. Intensity of Oestrus

Intensity of oestrus assessed using the scoring sheet is compared between the seasons in Table 4.28. Total number of oestrus detected with intensity assessment was 52, of which 23 were during DJF and lowest of only seven during JJA. Intensity of behavioral signs was very less during all the seasons with mean score of 2.25 out of 20. Scores of behavioral signs and ovarian changes had minimum variation between seasons.

Table 4.28. Mean scores for oestrus intensity assessed across the seasons

Chnages observed	**Items of observation**	**Maximum points**	**Season**				**Overall Mean**
			SON	**DJF**	**MAM**	**JJA**	
1	Behavioural changes	20	2	2.5	2.5	2	2.25
2	Breeding history	15	5.91	6.74	5.45	7.86	6.49
3	Mucous flow	15	7.18	9.29	6.82	5.57	7.22
4	Vulval oedema	5	3.45	2.74	3.27	2.71	3.04
	Vestibular hyperaemia	5	4.09	3.48	3.45	4	3.76
	Both **(External genitals)**	**10**	**7.55**	**6.22**	**6.73**	**6.71**	**6.80**
5	Cervical relaxation	10	8.64	8.74	8.82	9.43	8.91
	Uterine tonicity	15	10	12.83	10	10	10.71
	Both **(uterine changes)**	**25**	**18.64**	**21.57**	**18.82**	**19.43**	**19.62**
6	Medium / large follicle	15	10	13.26	15.91	13.57	13.19
	Corpus luteum	10	8.18	0.00	10	10	7.05
	Total **(Ovarian structures)**	**15**	**14.09**	**14.35**	**15**	**15**	**14.61**
	Grand total	**100**	**53.91**	**55.7**	**53.73**	**54.86**	**54.55**^{ns}
Total number of oestrus detected			11	23	11	7	13

^{ns} - Non-significant

Highest scores for breeding history, mucous flow, changes of external genitals and uterine changes were during JJA (7.86/15), DJF (9.29/15), SON (7.55/10) and DJF (21.57/25) respectively. Even though there were variations in the scores of individual signs between seasons, there was no similarity of the seasonal pattern and variation of the overall mean score was statistically non-significant.

4.2.7.3. Artificial Insemination

Details of the study groups, oestrus detected, AI done and non-return cases are shown in Table 4.29. Out of 38 inseminations done during the study period, five were among Gr.1 (n=22) and 33 among Gr. 2 (n=60).While 8 cows returned to service within 25 days of AI, cows not returned to service were 4 and 26 respectively among Gr. 1 and Gr. 2.

Monthly averages of oestrus detected and AI done among the study animals are compared between seasons in Table 4.30. Oestrus and AI were comparatively less during MAM and JJA and the highest were in DJF, even though the variations between seasons were statistically non-significant.

Table 4.29. Details of the study groups, oestrus detected, AI done and Non-return cases

Parameter	Group 1		Group 2		Total study group	
	Number	Per cent	Number	Per cent	Number	Per cent
Total animals selected	22	26.83	60	73.17	82	100
Total oestrus detected	11	17.46	52	82.54	63	100
Cows detected in oestrus	9	16.67	45	83.34	54	100
AI done/ total oestrus detected	5	45.45	33	63.46	38	60.32
Double AI out of total AI	2	40.00	19	57.58	21	55.26
Single AI out of total AI	3	60.00	14	42.42	17	44.74
Cows bred in single cycle	5	55.55	20	44.44	25	46.30
Cows bred in two cycles	0	0.00	5	11.11	5	9.26
Cows bred in three cycles	0	0.00	1	2.22	1	1.85
Cows bred / cows in oestrus	5	55.56	26	57.78	31	57.41
Return to heat	1	20.00	7	26.92	8	25.81
Non-retrun rate	4	80.00	19	57.58	23	60.53

Table 4.30. Monthly mean ± SE of oestrus detected and AI done compared betweenthe seasons

Parameter	Seasons				F - value	p- value
	SON	DJF	MAM	JJA		
Oestrus	5.67 ± 1.67	6.33 ± 1.76	4.33 ± 1.45	4.67 ± 1.45	0.33^{ns}	0.802
A I done	3.67 ± 1.67	4.33 ± 1.20	2.33 ± 1.45	2.33 ± 1.20	0.51^{ns}	0.684

ns - non-significant(P>0.05)

Correlation between oestrus detected and AI done with weather parameters are shown in Table 4.31. Oestrus detected was negatively associated with all the weather parameters studied, even though none of the correlations were significant. AI done showed a positive relationship with RH and negative relationship with temperature and THI even though all correlations were non-significant.

Correlation of oestrus detected and AI done with biological variables such as physical parameters (BCS, milk yield, feeding) and stress associated factors (HSP 70 and cortisol) are shown in Table 4.32.

Table. 4.31. Correlation of oestrus detected and AI done with weather parameters

Variables	Weather parameters				
	THI	MnT	MxT	MnRH	MxRH
Oestrus (p-value)	-0.270 (0.397)	-0.205 (0.524)	-0.252 (0.430)	-0.098 (0.763)	-0.135 (0.675)
A I done (p-value)	-0.266 (0.404)	-0.227 (0.478)	-0.188 (0.559)	0.023 (0.942)	0.061 (0.852)

All the correlations were non-significant (P>0.05)

Table 4.32. Correlation of oestrus detected and AI done with physical parameters and stress indicators

Variables	Physical parameters			Stress indicators	
	BCS	Milk yield	Feeding	HSP 70	Cortisol
Oestrus (p-value)	-0.34 (0.28)	0.038 (0.91)	-0.158 (0.625)	-0.20 (0.530)	-0.03 (0.93)
A I done (p-value)	-0.28 (0.37)	-0.02 (0.95)	-0.13 (0.70)	-0.20 (0.53)	0.02 (0.96)

All the correlations were non-significant (P>0.05)

4.2.8. Detailed Study

Monitoring of physiological parameters, stress indicators, ovarian function, endocrine profile, conception to AI and embryonic loss among selected cows

4.2.8.1. Cows Selected for Detailed Study

Every month eight cows (Gr. 2) of 28 – 91 days PP were subjected to detailed study and a total of 60 cows were includedduring the whole study period.

4.2.8.2. Respiration Rate and Body Temperature

Respiration rate and RT of the animals during the two seasons of high and low THI are given in Table 4.33. There was highly significant (P<0.01) variation between months as well as seasons with higher value in MAM compared to DJF. Pattern of monthly variations of RR and RT was almost similar and significant across seasons (P<0.01). Both RR and RT decreased in December followed by continuous increase until April and further onset of declining was noticed from May.

Table 4.33.Mean ± SE(n=104) of respiration rate and rectal temperature of the cows compared between the two seasons

Parameter	Seasons		t- value (p value)
	DJF	MAM	
RR (per min)	48.75 ± 1.38	64.82 ± 1.78	50.36** (P< 0.01)
RT (ºC)	38.50 ± 0.07	39.01 ± 0.05	29.50** (P< 0.01)

**. Significant (P<0.01)

Mean values of RR and RT at each time interval during the days of recording also showed highly significant variation (P<0.01) between the two seasons as shown in Tables 4.34 and 4.35. Irrespective of the seasons, mean RR decreased from 6.30 am to 9.00 am, there after elevated to reach the highest at 2.00 pm and declined thereafter.Between animals there was no significant variation for the mean values of these physiological parameters within either of the seasons

Table 4.34.Mean ± SE(n=104) of respiration rate at each time interval of recording compared between the two seasons

Recording time (Hours)	Seasons			t - value
	DJF	MAM	Both	
6.30	36.09 ± 1.16	52.58 ± 1.38	44.33 ± 1.07	9.14 **
9.00	32.3 ± 0.95	39.58 ± 1.03	35.94 ± 0.75	5.18 **
11.30	48.35 ± 1.24	66.89 ± 1.64	57.62 ± 1.21	9.01**
14.00	59.11 ± 1.22	81.08 ± 2.01	70.09 ± 1.4	9.33 **
16.30	57.02 ± 1.52	76.19 ± 1.94	66.61 ± 1.4	9.79 **
19.00	55.88 ± 1.28	73.6 ± 1.74	64.74 ± 1.24	8.20 **
Mean	48.12 ± 0.94	64.99 ± 1.27	56.55 ± 0.98	10.72 **

**. Significant (P<0.01)

Similar to RR, RT also dropped between 6.30 am and 9.00 am, increased thereafter, but the peak was attained by 4.30 pm deviating from that of RR and started decline only thereafter, irrespective of the seasons.

Table 4.35.Mean ± SE(n=104) of rectal temperature at each time interval of recordings compared between the two seasons

Recording time (Hours)	Seasons			t - value
	DJF	MAM	Both	
6.30	38.33 ± 0.05	38.72 ± 0.04	38.53 ± 0.03	6.48 **
9.00	38.16 ± 0.05	38.36 ± 0.03	38.26 ± 0.03	3.25 **
11.30	38.44 ±.0.04	38.83 ± 0.04	38.63 ± 0.03	7.19 **
14.00	38.74 ± 0.04	39.40 ± 0.05	39.07 ± 0.04	10.66 **
16.30	38.77 ± 0.06	39.59 ± 0.11	39.18 ± 0.07	6.60 **
19.00	38.54 ± 0.11	39.23 ± 0.05	38.88 ± 0.07	5.60 **
Mean	38.50 ± 0.04	39.02 ± 0.04	38.76 ± 0.03	9.38 **

**. Significant (P<0.01)

Besides direct correlation between each other, RR and RT showed significant positive correlation with many stress associated weather parameters as well as with HSP 70 as shown in Table 4.36. However, correlation of RR and RT with cortisol was found to be non-significant.

Table 4.36. Correlation of respiration rate and temperature with stress indicators and weather parameters

Parameter	Stress indicators		weather parameters						
	HSP	Cortisol	AvT	AvRH	THI	MnT	MxT	MnRH	MxRH
RR (Mean) (p-value)	0.291** (< 0.01)	0.062 (0.375)	0.553** (< 0.01)	0.329** (< 0.01)	0.636** (< 0.01)	0.650** (< 0.01)	0.148* (0.033)	0.346** (< 0.01)	-0.135 (0.052)
RT (Mean) (p-value)	0.228** (< 0.01)	0.086 (0.215)	0.423** (< 0.01)	0.318** (< 0.01)	0.513** (< 0.01)	0.535** (< 0.01)	0.058 (0.405)	(0.330)** (< 0.01)	-0.106 (0.127)

*. Significant (P<0.05) **. Significant (P<0.01)

4.2.9. Ultrasonography Findings on Ovaries

Each animal included for the detailed study were subjected to B mode USG at regular weekly interval starting from Day 28to 91 PP and ovarian structures especially follicles were observed as per the method described by Ginther *et al.* (1996). The findings were recorded with sequential numbering of scanning operations.

4.2.9.1. Follicular Growth

Count of different types of follicles detected on both the ovaries and categorized by subjective assessment of the appearance and ultrasonographic measurement of the diameter are compared between the seasons in Table 4.37.a. and mean number of large, medium and small follicles detected at each scanning are compared between seasons in Table 4.37.b.

Table 4.37.a. Mean ± SE of the count of different follicles types during the four seasons

Seasons	**Types of follicle (445 USG)**			
	Extra large (15-20 mm)	**Large (9-14 mm)**	**Medium (6 - 8 mm)**	**Small (3 – 5 mm)**
SON	1.67 ± 0.67	36.33 ± 6.67^{a}	34.67 ±7.80	114.33 ± 27.39
DJF	1.33 ± 0.67	40.00 ± 1.00^{a}	45.00 ± 1.00	160.00 ± 6.00
MAM	2.00 ± 1.15	61.00 ± 1.15^{b}	51.33 ± 7.69	163.00 ± 35.13
JJA	1.67 ± 0.67	42.67 ± 5.90^{a}	41.00 ± 1.53	145.33 ± 29.54
Total	1.67 ± 0.36^{NS}	45.00 ± 3.45*	43.00 ± 2.99^{NS}	145.66 ± 12.85^{NS}

*. Significant ($P<0.05$), F-value 5.911, ns - Non-significant

Means having different superscripts vary significantly across seasons

Table 4.37.b. Mean ± SE of different types of follicles detected at each USG examination during the four seasons

Season	Number of		Type of follicles			Mean ± SE Total number of follicles
	Scanning	Cows	Large (9-14 mm)	Medium (6–8 mm)	Small (3-5 mm)	
SON	93	17	1.17 ± 0.84^{a}	1.11 ± 0.12	3.69 ± 0.31	6.03 ± 0.31
DJF	106	21	1.06 ± 0.74^{a}	1.16 ± 0.12	3.91 ± 0.23	6.17 ± 0.27
MAM	129	20	1.42 ± 0.81^{b}	1.19 ± 0.11	3.79 ± 0.24	6.45 ± 0.26
JJA	117	22	1.09 ± 0.06^{a}	1.05 ± 0.11	3.72 ± 0.256	5.91 ± 0.30
Overall	445	80	1.12 ± 0.39**	1.13 ± 0.06^{NS}	3.78 ± 0.13^{NS}	6.16 ± 0.14^{NS}

Significant ($P<0.01$) NS – Non-significant

Numbers of extra large follicles were very few irrespective of the seasons. Medium and large sized follicles were more or less equal in number except during MAM, and small follicles were numerous during all the seasons with more numbers during MAM and DJF. Even though the count of different types of follicles varied significantly within the season (P<0.001), between seasons the difference was not significant except for more number of larger follicles during MAM (P<0.001). Mean number of total follicles per scanning was highest during MAM compared to other seasons, eventhough the difference was not significant statistically.

Correlation of the number of different types of follicles with THI and biological variables are shown in Table 4.38. There was no significant correlation of small and extra large follicles with any of the climatic or biological parameters. However, number of large sized follicles showed highly significant correlation with HSP 70 (P<0.001) and moderate association (P<0.05) with DM intake and BCS. Similarly medium follicle count was having moderate association (P<0.05) with BCS, HSP 70 and THI. At the same time mean count of any of the follicle types did not show significant correlation with other study parameters.

Table 4.38. Correlation of different follicle types with THI and biological parameters

Parameters	**Type of follicles**			
	Extra Large (15-20 mm)	**Large (9-14 mm)**	**Medium (6 - 8 mm)**	**Small (3 – 5 mm)**
BCS	0.175 (-0.586)	0.704 (-0.011) *	0.704 (-0.030) *	0.555 (-0.061)
Dry matter intake	0.260 (-0.414)	0.603 (-0.038)	0.466 (-0.127)	0.241 (-0.450)
Milk yield	0.407 (-0.190)	0.031 (-0.925)	0.273(-0.391)	-0.219 (-0.494)
Oestrus shown	-0.474 (-0.119)	-0.235 (-0.462)	0.310 (-0.327)	0.176 (-0.584)
Progesterone	0.046 (-0.888)	-0.543 (-0.068)	-0.142 (-0.660)	-0.011 (-0.974)
HSP70	0.250 (-0.434)	0.861 (<0.001)**	0.630 (-0.028)*	0.371 (-0.235)
Cortisol	0.512 (-0.089)	0.177 (-0.582)	0.013(-0.969)	-0.206 (-0.520)
THI	0.323 (-0.307)	0.551 (-0.063)	0.209 (-0.515)*	-0.0210 (-0.949)

* Significant (P<0.05) **. Significant (P<0.01)

4.2.9.2. Presence of Corpus Luteum

Presence of luteal structures categorized as functional CL (FCL) and regressing CL (RCL) based on ultrasonographic assessment of size, texture and blood flow characteristics arecompared across the seasons in Table 4.39.

Table 4.39.Mean ± SE of the numbers and size of different types of corpus luteum across the seasons

Parameter	Details of CLduring USG examination				
	SON	DJF	MAM	JJA	Overall
Number of FCL	12.33± 1.76	17.33±1.33	18.00±2.52	17.00± 3.79	16.17 ± 1.27
Size of FCL (mm)	17.43 ± 0.81	18.28 ± 0.69	17.98 ±0.41	17.57 ± 1.07	17.81± 0.35
Number of RCL	8.00 ±0.58	12.00± 1.00	10.33± 2.85	6.00± 1.73	9.18 ± 1.02
Size of RCL (mm)	10.16 ± 0.52	12.34 ± 0.59	11.40 ± 0.51	12.04 ± 1.23	11.48 ± 0.41

NS- variations non-significant between the seasons (P>0.05)

Between seasons, there was no significant variation for the type and size of CL. Also there was no significant correlation with weather parameters and other stress associated parameters. However, there was a positive association (P<0.05) between the presence of FCL as well size of RCL with number of small follicles. Similarly number of RCL was found to have positive association (P<0.05) with number of oestrus detected and AI done.

4.2.9.3. Ultrasonographic Examination during Oestrus

Numbers of follicles with a diameter of 8 mm or more were counted on both the ovariesduring Day 0 and Day 1of oestrus and major findings are compared in Table 4.40.

Table 4.40. Ultrasonographic findings ofovaries during Day 0 and Day1 of oestrus

Parameters	Day of oestrus	
	Day 0	Day 1
Total oestrus studied	34	34
Follicles of 8 mm or more	57	51
Single follicle of 8 mm or more	15	18
Size of the largest follicle (mm)	8 to 16	11 to 16
Size of the second largest follicle (mm)	8 to 14	8 to 12
Mean size of largest follicle (mm)	12.85± 0.36	13.56 ± 0.29
Mean size of second largest (mm)	9.68 ± 0.36	9.43± 0.28
Right ovary having the largest follicle	22	19
Left ovary having the largest follicle	12	14
Total follicles on right ovary	35	29
Total follicles on left ovary	22	22

Occurrence of larger follicles was slightly more during Day 0 (57 versus 51), whereas mean size of the largest follicle in mm was more on Day 1 (13.56) than Day 0 (12.85). However, size of the second largest follicle appeared to be slightly more on Day 0. More often right ovary was having the largest follicle irrespective of Day 0 or Day 1, so that total follicles detected on right ovary was 35 and 29 respectively on Day 0 and Day 1, as against 22 each on left ovary on both Day 0 and Day 1.

Details of follicles detected on Day 0 and Day 1 of oestrus are compared between four seasons in Table 4.41. There was no significant difference in the size of the largest follicle during oestrus across seasons. Correlation between size of follicles with other climatic and biological factors also showed no remarkable association between those factors.

Table 4.41.Ultrasonographic findings on Day 0 and Day1 of oestrus across seasons

Day of oestrus	**Parameters**	**Seasons**				**Overall**
		SON	**DJF**	**MAM**	**JJA**	
	Animals under study	10	13	5	6	34
Day 0	Total follicles	19	18	10	10	57
	Largest on Rt ovary	6	9	4	3	22
	Largest on Left ovary	4	4	1	3	12
	Size of the largest (mm)	13.1 ± 0.69	12.4 ± 0.59	13.4 ± 0.40	13.0 ± 1.15	12.85 ± 0.34^{ns}
Day 1	Total follicles	16	16	8	8	48
	Largest on Rt ovary	5	9	2	3	19
	Largest on Left ovary	4	4	3	3	14
	Size of the largest (mm)	12.9 ± 0.73	13.8 ± 0.38	13.8 ± 0.66	13.3 ± 0.61	13.45 ± 0.29^{ns}

ns – non-significant

4.2.9.4. Ultrasonography for Detection of Pregnancy

Inseminated cows of Gr. 2 not returned to oestrus within 25 days were subjected to USG for detection of early pregnancy and repeated USG at adequate intervals in those found to have or suspected for signs of pregnancy. A total of 64USG examinations were performed in 26 animals and details of scanning in each season are shown in Table 4.42.

Table 4.42.Ultrasonographic findings of early pregnancyacross seasons

Season	Number of scanning done for		
	Not conceived	Conceived	Total
SON	4	7	11
DJF	9	17	26
MAM	1	5	6
JJA	3	18	21
Total	17	47	64

4.2.9.5. Embryonic Death

In 19 animals found to have clear signs of conception at 25 days, USG was repeated at 10 days intervals to assess the conception loss. Four of the conceptions werefound missed at 35 days' USG and remaining 13 were found to be pregnant at 45 days USG as well.

4.2.10. Serum Sample Analysis

Serum samples collected and stored at the time of weekly scanning, on the days of oestrus (day 0 and 1) and at post-service intervals (Days 7, 14 and 21) were tested for HSP 70, cortisol, progesterone, oestrogen and MDA, as per the details given in Table 4.43. All the estimations were done in duplicate and the mean values were taken.

Table 4.43. Details of serum samples collected and components tested across seasons

Season	Collection details			Components tested for				
	Weekly Samples (A)	Oestrus Day 0 & 1 (B)	Post AI days 7, 14, 21(C)	HSP 70 A + C	Cortisol A	Progesterone A + C	Oestradiol B	MDA (A)
SON	94	20	30	124	94	123	20	0
DJF	105	26	38	143	105	143	26	105
MAM	129	10	14	143	129	143	10	129
JJA	117	12	17	134	117	134	12	0
Total	445	68	99	544	445	544	68	234

4.2.10.1. Stress Associated Biological Factors

Serum HSP 70, Cortisol and MDA were estimated in 544, 445 and 234 serum samples as shown in Table 4.43 to understand the biological response from exposure to TS.

i. Serum HSP 70 Level

Overall mean value of HSP 70 in 544 samples were 3.54 ± 0.16 ng /mL and values of individual samples ranged from 0.17to 31.99 ng/mL. After excluding 11 samples with extreme variation, the mean value of 533 samples was 3.06 ± 0.07 ng /mL.

Mean values of serum HSP 70 levels are compared between months, seasons and half years of long / short days and also rainy / non-rainy half years in Table 4.44. Between months, the highest level was during April and the lowest in September. Between seasons, there was marked increase during summer, compared to other three seasons. The variation of HSP 70 levels between months, quarters and half years were highly significant (P < 0.001).

Table 4.44.Mean ± SE of serum HSP 70 (ng/mL) across months, quarters and half years based on day length and raining pattern

Month	Samples	Minimum level	Maximum level	Periods			
				Months	**Quarters**	**Short/ long day half years**	**Rainy/ Non-rainy half years**
Sep	26	0.74	8.49	1.59 ± 0.30	2.09 ± 0.20^{a}	2.74 ± 0.14 (Short days)	2.56 ± 0.12 (Rainy)
Oct	50	0.17	22.69	2.40 ± 0.46			
Nov	48	0.71	3.31	2.05 ± 0.11			
Dec	45	0.85	3.64	2.48 ± 0.12	2.63 ± 0.07^{ab}		4.42 ± 0.28 (Non-rainy)
Jan	63	0.91	4.76	2.65 ± 0.11			
Feb	35	1.51	4.78	2.79 ± 0.14			
Mar	34	2.06	29.13	5.53 ± 0.81	6.24 ± 0.51^{c}	4.52 ± 0.30 (Long days)	
Apr	47	1.02	31.99	7.04 ± 1.05			
May	61	1.24	27.14	6.02 ± 0.75			
Jun	44	1.30	8.46	3.40 ± 0.27	2.98 ± 0.13^{b}		2.56 ± 0.12 (Rainy)
Jul	41	1.57	6.45	2.69 ± 0.18			
Aug	50	0.97	7.66	2.85 ± 0.19			
Overall	544	0.17	32.00	3.54 ± 0.16	F value = 42.10** (P<0.001)	F value = 32.28** (P<0.001)	F value = 35.84** (P<0.001)

Means with differentsuperscripts varied significantly between quarters (P<0.001)

ii. Serum Cortisol Level

Overall mean value of serum cortisol in 445 samples was 8.27±0.20 ng/mL. Mean values of cortisol levels are compared between months, seasons and half years of long / short days and also rainy / non-rainy half years in Table 4.45.

Serum cortisol also varied significantly between seasons with the highest level attained during MAM, but the patterns during other seasons were different from that of HSP 70. Accordingly, there was no significant correlation between HSP 70 and cortisol. Correlation of HSP 70 and cortisol was also compared with every other weather parameters and biological parameters and are already described under respective sections.

Table 4.45.Mean ± SE of serum cortisol (ng/mL) across months, quarters and half years

Month	Minimum	Maximum	Periods			
			Months	Quarters	Short/ long day half years	Rainy/ Non-rainy half years
Sep	5.02	19.48	8.07 ± 0.6	8.84 ± 0.30^{bc}	7.79 ± 0.36 (Short days)	8.26 ± 0.22 (Rainy seasons)
Oct	5.13	16.51	9.14 ± 0.52			
Nov	4.49	14.68	9.03 ± 0.46			
Dec	2.71	13.56	5.37 ± 0.34	6.84 ± 0.62^{a}		8.28 ± 0.32 (Non-rainy season)
Jan	2.63	64.59	8.76 ± 1.53			
Feb	3.19	9.84	6.05 ± 0.33			
Mar	5.5	19.34	11.49 ± 0.58	9.44 ± 0.25^{c}	8.66 ± 0.21 (Long days)	
Apr	4.74	16.43	9.53 ± 0.38			
May	5.05	14.39	8.0 ±0.28			
Jun	5.89	17.13	9.64 ± 0.47	7.80 ± 0.33^{ab}		8.26 ± 0.22 (Rainy seasons)
Jul	5.17	15.33	9.31 ± 0.4			
Aug	1.54	17.09	4.81 ± 0.47			
Total	1.54	64.59	8.27 ± 0.2	F value = 0.72** (P<0.001)	F value = 4.73** (P<0.05)	F value = 0.002, (P value = 0.936)

Means having different superscripts varied significantly between seasons (P<0.001)

iii. Serum MDA Level

MDA levels of serum (µ.moles/mL) were estimated during post monsoon and summer seasons and are compared in Table 4.46. The mean values varied significantly with the highest value in summer (P<0.001).

Table 4.46.Mean ± SE of serum MDA level (µ.moles /ml) of the two seasons

Season	Mean	t-value
DJF	2.46 ± 0.007	8.324**
MAM	3.82 ± 0.136	(p<0.001)

**. Significant (p<0.001)

Correlations of MDA levels with weather parameters are shown in Table 4.47. There was significant positive correlation of MDA with AvT, THI, MnT and MnRH.

Table 4.47. Correlation coefficient of MDA with weather parameters

Parameter	Weather parameters						
	Temp	RH	THI	MnT	MxT	MnRH	MxRH
MDA	0.339**	0.140*	0.365**	0.410**	0.072	0.156*	-0.099
(p-value)	(< 0.001)	(0.032)	(< 0.001)	(< 0.001)	(0.275)	(0.017)	(0.129)

*. Significant (P<0.05); **. Significant (P<0.01)

Correlations of MDA with biological variables are shown in Table 4.48. All the parameters showed highly significant (P< 0.001) positive correlation with MDA.

Table 4.48.Correlationcoefficient of MDA with biologicalvariables

Parameter	Biological variables			
	Mean RR	Mean RT	HSP 70	Cortisol
MDA	0.280**	0.307**	0.200**	0.315**
(p-value)	<0.001	<0.001	0.002	<0.001

**. Significant (P<0.01)

4.2.10.2. Reproductive Hormones

i. OestradiolLevel

Oestradiol level (pg/ mL) on Day 0 and Day 1 of oestrus during the four seasons and overall mean are compared in Table 4.49. There were significant differences in the E2 levels of day 0 (P <0.01) and day 1 (P <0.05) between the seasons, the highest being during JJA and lowest during SON irrespective of the days of collection.

Table 4.49.Mean ± SE oestradiol levels (pg/mL) on Day 0 and Day 1 of oestrus acrossseasons

Seasons	E2 levels (pg/mL)	
	Day 0	Day 1
SON	31.9 ± 5.1^a	32.8 ± 6.0.^{a}
DJF	51.6 ± 4.6^b	46.5 ± 6.0^{ab}
MAM	52.8 ± 9.1^b	49.6 ± 3.8^{ab}
JJA	62.1 ± 5.6^b	61.9 ± 4.3^b
Over all	47.8 ± 3.31	45.7 ± 3.43
F-value (p-value)	4.89** (0.007)	3.34* (0.032)

*. Significant (P<0.05) **. Significant (P<0.01)

Values with different superscripts varied significantly within the columns

ii. Serum Progesterone Level

Progesterone levels were estimated in serum samples collected post AI to assess the luteal phase P4 level and in weeklysamples collected at USG,for the basal level of P4.

Table 4.50.Mean ± SE serum progesterone levels during postpartum period in the four different seasons

Season	Mean progesterone level (ng/ml)
SON	0.72 ± 0.16
DJF	0.56 ± 0.19
MAM	0.36 ± 0.14
JJA	1.01 ± 0.18
Overall	0.67 ± 0.10
F-value 2.55^{ns}; p-value = 0.129	

ns – non-significant

a. Postpartum Progesterone Level

Progesteronelevels of serum collected at weekly intervals during the PP period are compared between the four seasons in Table 4.50. Lowest level assessed (0.36 ng/mL) was during MAM and the highest (1.01 ng/mL) during JJA, even though the variation was non-significant between seasons. Between half years, P4 level was significantly high (P<0.05) during rainy than non-rainy seasons. Basal P4 level had significant negative correlation with

MxT ($P<0.05$) and number of medium sized follicles ($P<0.01$). However, no significant association of basal P4 level was found with HSP 70, cortisol and other study parameters.

b. Post-service Progesterone Level

Progesterone levels of the three post-service intervals are compared between the seasons in Table 4.51. Overall mean P4 level was less at 7th day of cycle (0.84 ng/mL), compared to the levels of 14thday (0.99 ± 0.16 ng/mL) and 21st day (0.97± 0.13 ng/mL) and the variations between seasons were statistically non-significant at all the three intervals.However,overall average of the three intervals showed significant variation ($P<0.05$) between seasons with the highest and lowest during DJF and SON respectively.

Post-service P4 level did not show significant correlation with any of the weather, physical and biological factors including HSP 70, cortisol and MDA, except the association with the presence of a recent RCL.

Table 4.51.Comparison of mean ± SE serum progesterone levels (ng/mL) during various post-serviceperiods in four different seasons

Season	Mean ± SE serum progesterone levels (ng/mL)							
	Day 7 (n=33)		Day 14 (n=33)		Day 21 (n=32)		Total (n=98)	
SON	10	0.59 ± 0.06	10	0.68 ± 0.05	9	0.73 ± 0.04	29	0.65 ± 0.03^{a}
DJF	13	1.12 ±0.29	13	1.22 ± 0.34	12	1.19 ± 0.32	38	1.18 ± 0.18^{b}
MAM	5	0.90 ± 0.31	4	1.47 ± 0.74	4	1.07 ± 0.28	13	1.13 ± 0.25^{b}
JJA	5	0.52 ± 0.08	6	0.70 ± 0.06	7	0.92 ± 0.22	18	0.73 ± 0.09^{a}
Total	33	0.84 ± 0.13^{ns}	33	0.99 ± 0.16^{ns}	32	0.97 ± 0.13^{ns}	98	0.93 ± 0.08*
F-value (p-value)		1.376 (0.270)		1.186 (0.332)		0.590 (0.627)		3.124 (0.030)

ns. Non-significant; *. Significant ($P<0.05$); Means with different superscripts vary significantly

Out of the 33 inseminations among Gr. 2 cows, 17 resulted in conception. Comparison of P4 levels between conceived and non-conceived animals across the seasons are shown in Table 4.52.

Mean P4 level of serum samples collected at three stages of post-service period showed significant ($P<0.05$) difference between conceived and non-conceived animals (1.10

± 0.13 and 0.70 ± 0.03 respectively). The variation of mean P4 levels among conceived animals across seasons was highly significant ($P<0.01$) with the higher levels during non-rainy (1.59 ng/mL) than rainy (0.72 ng/mL)seasons. Mean P4 levels of non-conceived animals also showed higher levels during non-rainy (0.71 ng/mL) than rainy (0.50 ng/mL) seasons, though the variation was non-significant.

Table 4.52.Mean ± SE serum progesterone levels (ng/mL) in post-servicesamples compared between conceived, not conceived and total animals in four different seasons

Season	**Mean ± SE serum progesterone levels (ng/mL)**		
	Conceived (n=57)	**Not Conceived (n=41)**	**Total (n=98)**
SON	0.70 ± 0.04^{a}	0.61 ± 0.05	0.65^{a}
DJF	1.61 ± 0.34^{b}	0.79 ± 0.07	1.18^{b}
MAM	1.56 ± 0.41^{b}	0.62 ± 0.07	1.13^{b}
JJA	0.73 ± 0.09^{a}	0.49 ± 0.00	0.73^{a}
Total	1.10 ± 0.13**	0.70 ± 0.03^{ns}	0.93 ± 0.08*
F-value (p-value)	1.298 (0.009)	2.477 (0.980)	3.124 (0.030)

*. Significant ($P<0.05$) **. Significant ($P<0.01$) ns – non-signifcant

Means with different superscripts varied significantly within the columns

4.2.11. Pregnancy Diagnosis

Out of the 33 animals inseminated among the Group 2, 5 returned to service before 25 days of AI. In a total 64 scanning, 10 were found to be non-pregnant at the first check itself and another 4 more detected in the course of repeated scanning. Out of the 18 pregnant at 25 days, ten needed recheck after 2 days to make sure of the pregnancy changes.

4.2.11.1. Conception Rate of AI

Conceptions obtained out of the total 38 AI including 5 in Gr. 1. (cows not included for detailed study), during each of the seasons are shown in Table 4.53.The numbers of inseminations were very low especially during MAM and JJA (seven each). Number of inseminations and conceptions were more or less similar during SON and DJF. The highest conception rate was obtained in JJA (85.71%) and lowest in MAM (28.57 %), even though the variations were non-significant between seasons.

Table 4.53. Conception among the cows bred in four seasons

Season	Total Bred	Conception	
		Number	Per cent
SON	11	5	45.45
DJF	13	6	46.15
MAM	7	2	28.57
JJA	7	6	85.71
Total	38	19	50.00
Chi square = 2.641 ^{ns}, p value = 0.450			

Ns – Non-significant

4.2.11.2. Pregnancy Loss

Embryonic loss detected at scanning and pregnancies confirmed are compared between seasons in Table 4.54.

Table 4.54.Conceptions, embryonic loss and pregnancies confirmed during the four seasons

Season	Animals Conceived	Pregnancy loss				Pregnancy confirmed	
		25-35 d	35-45 d	Total	%	Number	%
SON	5	2	0	2	40.00	3	27.27
DJF	6	1	0	1	16.67	5	38.46
MAM	2	0	0	0	0.00	2	28.57
JJA	6	1	0	1	16.67	5	71.42
Total	19	4	0	4	21.05	15	39.47

All the four embryos lost were between 25 and 35 days of pregnancy. Embryonic loss was more during SON and none during MAM. Numbers of conceptions were more or less equal during all the seasons except MAM (only two), even though total number was less.

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DISCUSSION

5. DISCUSSION

. Climate change is expected to becomeone of the major threats for animal husbandry as it causes multi-dimensional impact on productivityof animals. Elevation of AtTis going to be the major climatic factor causing maximum adversities on health and well being of the animals especially dairy cattle (Marai *et al.,* 2008; Prasad *et al.,* 2013). Increase of milk production achieved through well focused breeding approaches has made dairy cattle highly vulnerable to elevation of AbT. Consistent increase of milk production has resulted simultaneous elevation of metabolic heat production (Schuller *et al.,* 2014) necessitating more dependence on heat dissipation mechanisms to maintain the thermal equilibrium (Gaughan *et al.,* 2013).

Elevation of AtTcaused by climate change is projected at the rate of 0.2 ºC per decade (Sejian *et al.,* 2013b; Das *et al.,* 2016)and is going to affect very much on the passive dissipation of excess heat, making the animal under TS very often (El-Tarabany and El-Tarabany, 2015). In response, besides initiation of active heat dissipation mechanisms at the expense of energy reserves (Singh and Singh,2005), long term measures will be to cut down the non-essential physiological processes especially reproduction (Polsky and Von-Keyserlingk, 2017). Thus affection of reproductive performance will be the major impact of climate change on animal production (De-Rensis*et al.,*2015).

It is already established that reproductive rate of dairy animals is declining simultaneous with the hike of milk production (De-Rensis *et al.,* 2017; Krishnan *et al.,* 2017). Reproductive rate of dairy animals in tropical countries is already low (Das *et al.,* 2016). In addition, climate change is going to have serious impact at first in tropical countries; due to the prevalence of high AtT(Sejian *et al.,* 2013b). Thus it is highly essential to study the current influence of TS especially on the reproductive performance of dairy animals and its impact so far over the years. Hence, the present study focused on reproductive performance in the previous few years together with prospective assessment of the present day impact of TSon animal fertility over a period of one year.

a. Study Location

The dairy farm located at Thiruvazhamkunnu in Palakkad district was selected for the study due to the warmest climate than other districts of Kerala (Rao, 2013), prevailing wind following the rainy season causing considerable drying of the soil and presence of rocky mountains in the vicinity contributing to the elevation of AbT especially during summer. Availability ofa dairy cattle herd comprised of around 300 crossbred cattle maintained since long time and other facilities for research formed other favourable factors in selecting the locality for the study.

b.Climatic Features

Comparatively high AbT of the locality with MxT of the summer reaching around 40ºC, MnT also maintained at a higher level and wide diurnal variation of the AbT makes the climate highly stressful to the animals especially during the summer. Moderately high annual rainfall causing elevation ofRHeven to the point of saturation many days during the rainy season and intermittent rainfall of non-rainy seasons contributing high humidity together with rise of temperature makes the animals highly uncomfortable during these periods. Further, more than extremes of the climate, the inconsistency of the weather across the years caused by climate change phenomenon is becoming serious concern in planning and handling the impact of TS on dairy animals.

c. Seasons in Kerala

Classification of seasons in the present study was done as four quarters of three months each, similar to an earlier study on seasonality of reproduction in goats (Kutty, 1995; Kutty, 2013). Seasons in Kerala is often classified with four months under Southwest monsoon (June to September), two months under North East monsoon (October and November) and three months each under winter (December to February) and summer (March to May). This classification is mainly based on the occurrence of monsoon rain fall and direction of wind causing the rain (Kumar, 2013; Rao, 2013).

September is the month of equinox, with equal duration of day and night at the equator. In Kerala, climate of September is having more similarity to that of October-November period with respect to AtT, amount of rainfall, day length, hours of sunshine, atmospheric humidity and THI compared to the previous period of June to August. Since forth-mentioned climatic factors are more influential on reproductive processes of the animals, the month of September was considered together with October and November in formulating the operational classification of seasons used for the present study as four quarters of three months each as used in the earlier study (Kutty, 1995).

The period of December to February forms the winter season in rest of the country (Kumar, 2013). However, Kerala is lacking a winter climate except in high ranges and hence the period of DJF is designated as post monsoon to denote the climate that prevails in most part of the state including the study location where in the lowest temperature recorded across the year was 18.3ºC.

d. Animal Stock, Housing and Management

The crossbred cattle maintained in the farm were produced out of inter-breeding local non-descript cattle with exotic breeds such as Jersey, HF and BS. Considering the prevailing warmer climate, Jersey breed is given preference in the current cross breeding strategy of the farm for having better climate adaptability and management convenience rather than solely for high production. Thus available animals are a mixture of predominantly Jersey together with other breeds such as HF and local non-descript and least involvement of BS. Accordingly, the animals are of lesser body weight and moderate milk production compared to the cross bred cattle maintained under other research farms of Kerala Veterinary and Animal Sciences University (Prasad, 2014; Harikumar, 2017).

5.1. RETROSPECTIVE STUDY

Retrospective data collection enabled the assessment of reproductive performance of female cattle maintained in the farm over a period of six years starting from September 2013. The period was decided mainly based on the availability of farm records and weather data and also to unify with the period of prospective study, *ie;* September 2018 to August 2019. In addition, September forms the period of transition between the seasons in most parts of the country (Kumar, 2013) and in Kerala September is the interim period between Southwest and North East monsoons, with sparse rainfall and lot of variability in the pattern of rain fall between years (Rao, 2013).

Photoperiodicity forms one of the major regulators of reproductive process (Kilgoura *et al.,* 2012; De-Rensis*et al.,* 2017) and shifting from long day to short day period at the equatorial region occurs during September (Autumn equinox), and is effected few weeks earlier at the Kerala latitude (8° 25' to 12° 75' N). Also September formed the period of moderate weather conditions with respect most of the variables and the period of better green grass availability. Thus, onset of the study was selected at the optimum period for weather based studies in this region.

5.1.1. Weather parameters in the Past Six Years

Monthly trends of mean AbTof the six years in ºC are shown in Fig. 5. 1. Both AvT and MnT showed a steep rise from the lowest to highest in a matter of three months. MxT had its lowest monthly average in July (29.70) and attained the highest average during March (37.04). Even though day length is the primary regulator of MnT and MxT as seen in other parts of the country, early occurrence of rain fall in Kerala (Rao, 2013) caused a major drop in AtT starting from April, changing the pattern of MxT irrespective of the day length and the same resulted in the lowest MxT and AvT respectively during June and July.

As shown in Table 4.1 quarterly means of daily AvT and MnT had almost the same pattern of variations, whereas MxT showed a consistent increase from JJA to MAM and a subsequent drop to the lowest in JJA (Fig. 5.2), attributable to the effect of monsoon rainfall. Thus, even though the highest averages of all the types of temperatures were in summer (MAM), the lowest of MxT and AvT was in JJA and only MnT had the lowest mean value in DJF. This in turn makes it very clear that there is no winter season in the study locality.Hence, the usage of the term post monsoon is well substantiated, deviating from the usual description of DJF as winter in other parts of the country with clear cut climatic features, especially the lowest of AtT (Kumar, 2013).

The pattern of MnT and MxT as shown in Fig. 5.1 showed increasing difference between the two starting from December, attaining maximum in January, maintains almost same level until March and there after declined. Comparing the two half years of rainy and non-rainy seasons, there is significant difference ($P<0.001$) between MnT and MxT, with more difference during non-rainy than rainy season. The range of variation between MnT and MxT appears to have direct association with the day length since shortest day length is during December (Winter solstice) and increases thereafter to equalize the duration of day and night hours during March (Spring equinox). Even though the difference between MnT and MxT started to reduce from March, the rate of reduction was very slow until June - the period of longest days (Summer Solstice) and there after declines faster until September (Autumn equinox) and start to increase again thereafter.

The trend of mean RH for the six years (Fig. 5.3)showedsignificant variation between months with the lowest mean value (77.37 %) during February and the highest (97.75 %) in July. It appears that the RH is associated with rain fall, since the two months

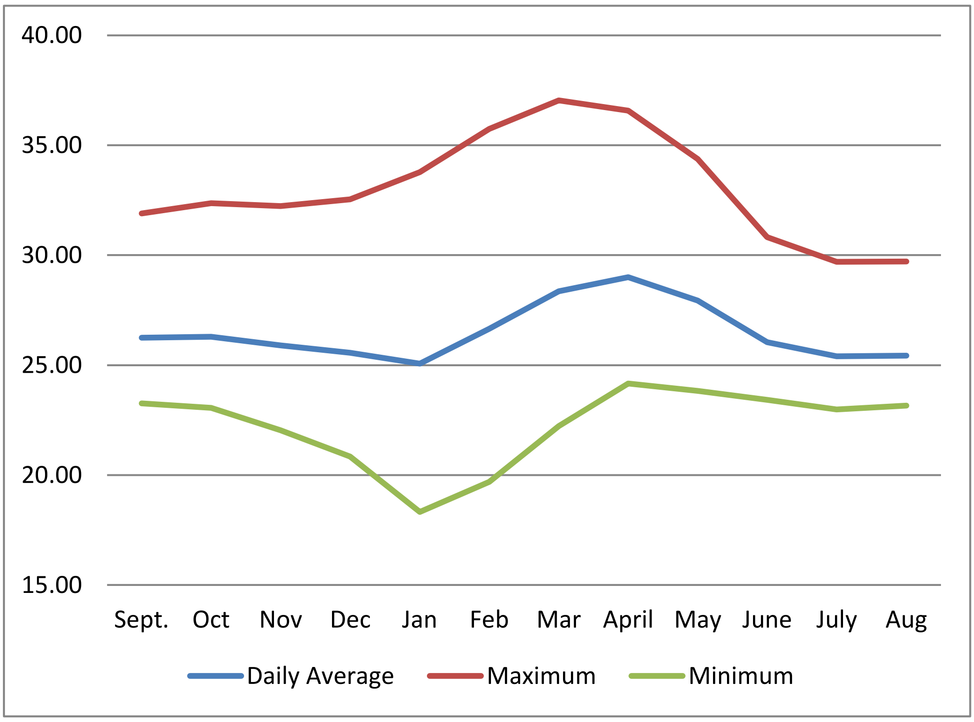

Fig. 5.1. Monthly mean ambient temperature of six years in °C

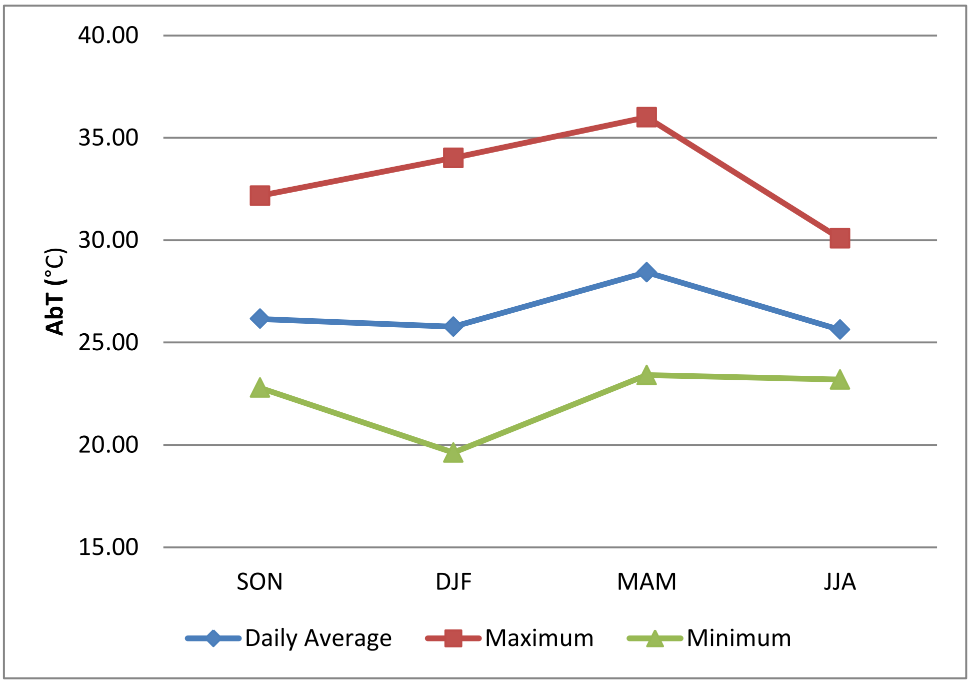

Fig. 5.2. Quarterly means of ambient temperature for sixyears

of lowest and highest RH corresponds respectively the periods of lowest and highest rainfall in the region. Correspondingly, quarterly means of all the three measurements of RH (Table 4.1) showed similar pattern of variation across seasons with the lowest in DJF, increased therafter to reach the highest in JJA and decreased again towards the lowest in DJF.

AbTforms the main contributor of TS and is precipitated by elevation of RH as it interfereeevaporative cooling mechanisms of the body(Polsky and Keyserlingk, 2017). Hence, simultaneous elevation of both becomes more stressful to the animals. However, it is less likely under climatic situation of Kerala since rainfall regulates both these variables in an opposite manner. This is evident in this study as well since the period of highest RH (97.75 %) happened to be during July which is the period of lowest mean for MxT (29.70 ºC). Likewise, RH is lowest in February while highest mean of MxT is during the adjoining months such as March (37.04 ºC) followed by April (36.57ºC) and February (35.74 ºC). Thus, besides extremes of AbT and RH, moderate elevation of both these variables and their different combinations also produces more stress in animals. Thus a composite measurement of AbT and RH becomes more meaningful in the context of TS.

Monthly trend of mean THI (LPHSI)for the six years (Fig. 5.3) is compared with that of RH and AbT in Fig 5.3 and 5.4 respectively. The pattern of THI variation across months had more similarity with that of AvT than RH, indicating more influence of AvT to the THI than RH.Across seasons, highest mean value for daily AvRH was 97.32 per cent during JJA and lowest of 82.61 per cent during DJF. As shown in Table 4.2,THI value based on the daily means of AbT and RH was highest during MAM (81.36) and lowest of 76.42 during DJF. Thus, quarterly averages of THI and AbT followed same pattern, whereas the pattern was different for RH.

Highest mean value for THI was observed during MAM – the quarter of high AbT (28.43ºC) and moderate RH (86.98 %), while lowest of mean THI was during DJF characterized by lowest RH (82.61 %) and low AbT (25.76 ºC). THI was moderate during JJA (the quarterof highest RH – 97.32and lowestAbT - 25.62 ºC) as well as SON (with moderate RH and AbT such as 94.54 per cent and 26.15 ºC respectively) as shown in Table 4.1 and 4.2. Thus, combinations of moderate AbT and RH contributes TS more often than higher values of AbT and RH, since occurrence of both together is less likely in Kerala climate (Kutty, 2013; Rao, 2013).

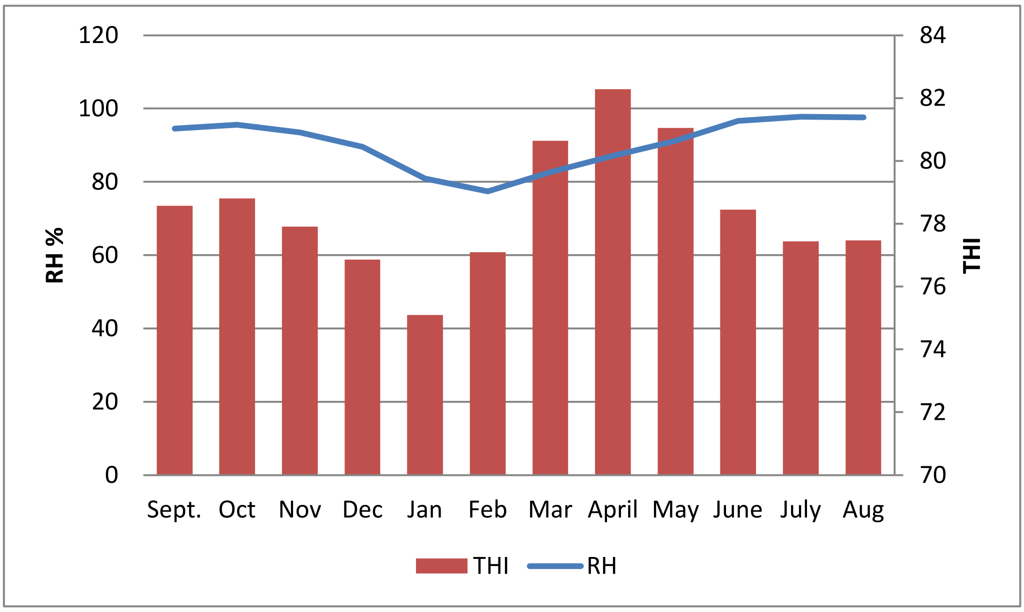

Fig. 5.3. Monthly means of RH and THI during the six years

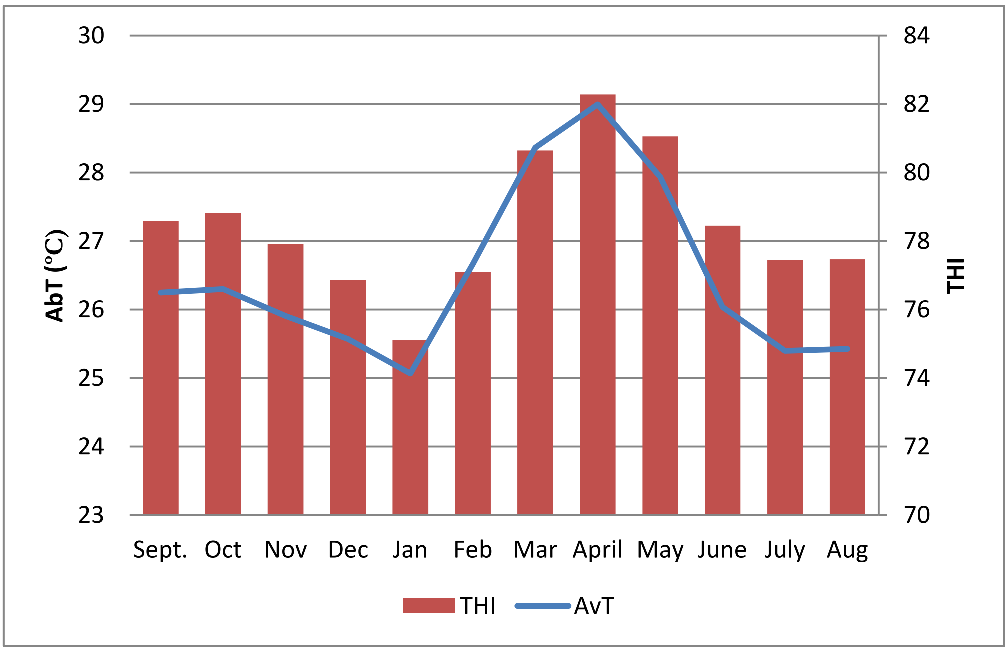

Fig. 5.4.Monthly means of AvT and THI during the six years

In order to understand all the possible ranges of THI variation in the prevailing climate, THI was calculated using the daily maximum and minimum values for AbT and RH during all the seasons as shown in table 4.2. Mean values of THI based on minimum values daily AbT and RH exceeded 71 in three seasons and the lowest possible THI based on maximum values AbT and RH did not fall below 83 in any of the seasons indicating persistence of rather high THI to cause mild to moderate TS throughout the year.

There exists an inverse relationship (-0.637, p-value 0.001) between the maximum recordings of temperature and humidity, so that maximum of AbT and RH of any particular day will not coincide. Hence THI calculated based on daily average of weather parameters becomes the relevant one.Range of monthly mean THI based on minimum and maximum values of AvT and AvRH varied from 75.87 in DJF to 82.51 in MAM indicating prevalence of mild to moderate stress throughout the year.

Since the lowest THI value based on monthly means of AbT and RH(75.87) exceeded the THI level prescribed for thermal comfort of dairy cattle (68 to 72), it can be inferred that the animals are exposed to mild to moderate level of TS throughout the year, irrespective of the months or seasons. THI remained within the level for mild stress (72 to 79) most of the seasons and even elevated to the level for moderate stress (80 to 89) during summer (MAM). However, THI of the study locality in any of the seasons did not reach the level for severe stress (90 or more)during the period of study.

5.1.2.Herd Strength

Comparison of animal stock under different breeding categories (Table 4.3) did not show significant variation between seasons, even though there was highly significant variation ($P<0.01$) between the years (Table 4.9 and Appendix 1.). This can be due to the alterations in management strategies between years and even between months of different years so that pattern of each variable with different monthly mean values across the years leads to nullification of the variation while computing the quarterly means.

5.1.3. Breeding Activities

Most of the reproductive parameters (Table 4.4.) showed variation between seasons with the lowest performance during summer months though the differences were non-significant statistically. Most of the earlier studies concurs the finding of poor reproductive performance during summer and attributed the same to TS influence (Wolfenson *et al.*, 2000; De-Rensis and Scaramuzzi, 2003; Hansen, 2009; Kumari and Pampana, 2015). However non-significant variation can be attributed to the lack of prominent seasonality and consistency of general management across the months.

In the present study, conception rate of AI was slightly better during summer (Table 4.4), contrary to the earlier reports (Bouhroum *et al.*, 2014; Das *et al.,* 2016), which might be due to reduced number of oestrus detected (Table 4.5.) concurring the report by Sonmez *et al.* (2005), so that only those few animals having prominent signs will be detected and inseminated leading to better conception rate during summer. This is further evidenced by the lowest number of conceptions even though conception rate was highest during MAM.

Breeding management related parameters (Table 4.5.) also showed non-significant variation between seasons except for the DAI conception rate. DAI was performed as a management measure for prolonged oestrus and formed 26.0 per cent of the total oestrus cycles in which AI was done. Irrespective of the seasons, DAI yielded better conception rate (42.6 %) than single AI (34.0 %). Conception rate from DAI showed significant variation ($P<0.05$) with the lowest rate of 31.5 per cent during JJA and the highest during SON (51.00 %). This can be attributed to overall lowered conception rate during JJA probably contributed by poor quality of oocyte and altered endocrine profile after the summer (Torres-minor *et al.,* 2008;Abdalla *et al.,* 2017; Kristyna *et al.,* 2017).

Highest conception rate of DAI was during SON, probably contributed by the favourable climate and availability of good quality green fodder. However, conception rate of single AI was less during SON (30.4 %) compared to DJF (39.1 %). This might be due to the prevalence of more prolonged oestrus during the period of more lush green fodder so that DAI is more benefitted than single AI. Highest conception rate of single AI during DJF can be due to less number of animals exhibiting prolonged oestrus. Having cleared off the repeat breeders from summer and earlier months during JJA and SON, inclusion of more new animals under breedingmight have contributed better conception to AI, indicated by comparatively lesser number of total AI done during DJF than SON.

Highest number of DAI was during DJF (32.0 %) with slightly lesser success rate (47.1 %) than SON,since DAI was mostly done for problem animals not conceived during the previous months and were found to exhibit prolonged oestrus.Double AI conception during the summer (40.9 %) was significantly ($P<0.05$) better than the same during JJA (31.5%), which can be attributed to detection of only those animals with prominent and prolonged signs (Singh *et al.,* 2012). During JJA conception rate of DAI was much less and even below that of single AI (32.2 %), indicating more influence by delayed consequences of TS during JJA as reported by Sonmez *et al.*(2005).

5.1.4. Fertility Indices

Comparison of fertility indices (Table 4.6) over the six years periodalso showed no significant variation between seasons. Parameters such as onset of PP oestrus, service period and calving to conception interval showed best performance for the animals calved during MAM, which may be due to the favourable season that followed summer, with respect to climate and green grass availabilityso that the prolongation of fertility parameters due to intervening summer gets avoided.

Number of calvings per adult females was highest (Table 4.4) during SON and JJA compared to other two seasons and was corresponding to more number of animals conceived during previous DJF and SON. These two periods were having the lowest and moderately high THIrespectively, favouring better conception. But more than THI, indirect effects of lowered conception during MAM and JJA caused by direct and delayed effect of TScan be attributed to the increase of conception in DJF and SON. Adverse influence of TS on fertility has been found to persist for around 3.5 months (Torres-minor *et al.,* 2008; De-Rensis *et al.,* 2015; Krishnan *et al.,* 2017) so that more number of breedable animals carried over from MAM gets conceived in SON and DJF owing to better climate and lower THI.

5.1.4.1. Variation between High and Low THI Periods

Between the two periods of low (72 to 78) and high THI (more than 78) there was non-significant variation for the breeding stock categories, reproductive performance and fertility related parameters. This is in contrary to many earlier reports of fertility reduction with THI increase (El-Tarabany and El-Bayoumi, 2015; Krishnan *et al.,* 2017) and can be attributed to the type of animals, since crossbred cows are less affected as against purebreds and adaptation of the herd due to continuous exposure over many years. El-Tarabany and El-Tarabany (2015) reported significantly longer calving interval and days open at high THI as against low THI period in Hostein cows, where as there was no such difference in crossbred cows for the same parameters between the two periods of low and high THI.

5.1.5. Seasonality of Reproduction

Even though well defined seasons comparable to temperate region is lacking in the tropical climate, seasonal pattern of reproductive performance has been well established among farm animals (Kutty, 2005; Sonmez *et al.*, 2005). Various factors have been reported as determinants of the seasonal variations (Bouhroum *et al.*, 2014), major one being TS (Wolfenson *et al.,* 2000; Collier *et al.*, 2017)influenced by AbT and RH (Kutty, 2005; De-Rensis, *et al.,* 2017). Pattern of monthly and yearly variations of AbT, RH and THI over the period of six years are shown for comparison in Figures 5.5 to 5.7. Even though the pattern of monthly variation was very distinct between seasons for AbT, RH and THI, across the years there was no distinct pattern with respect to all these weather parameters.

5.1.5.1. Fertility Variation between Seasons

Between seasons, there was no significant correlation between reproductive parameters and weather parameters such as AbT and THI. However, RH showed significant negative or positive correlation ($P<0.05$) with fertility parameters as shown in Table 4.7. These correlations appeared to be incidental since there was no uniformity of pattern for three levels of RH; and THI calculated from these RH values did not show significant correlation with any of the reproductive and breeding related parameters. Thus, lack of significant variation of most of the fertility parameters between seasons appeared to be due to inconsistency of the monthly pattern contributed by variations in management and climatic factors across the years so that the variability gets minimized upon comparison of seasons across few years. Adaptability of the animal to maintain the performance without much variation irrespective of the weather can also be another possibility.

Between seasons, different fertility parameters showed significant positive or negative correlation with each other. To illustrate, as the conception rate of total AI or single AI per cycle increased, total number of AI decreased. Also with the double AI proportion increase, number of oestrus cycles inseminated decreased; as the number of AI increased, proportion of pregnant animal became less and so on. Thus, all the negative correlations shown in Table 4.8 indicated the mutual dependence of different fertility related variables.

5.1.5.2. Fertility Variation between Years

Even though the mean values of weather parameters showed significant variation between months, the variation was non-significant between years indicated by almost similar

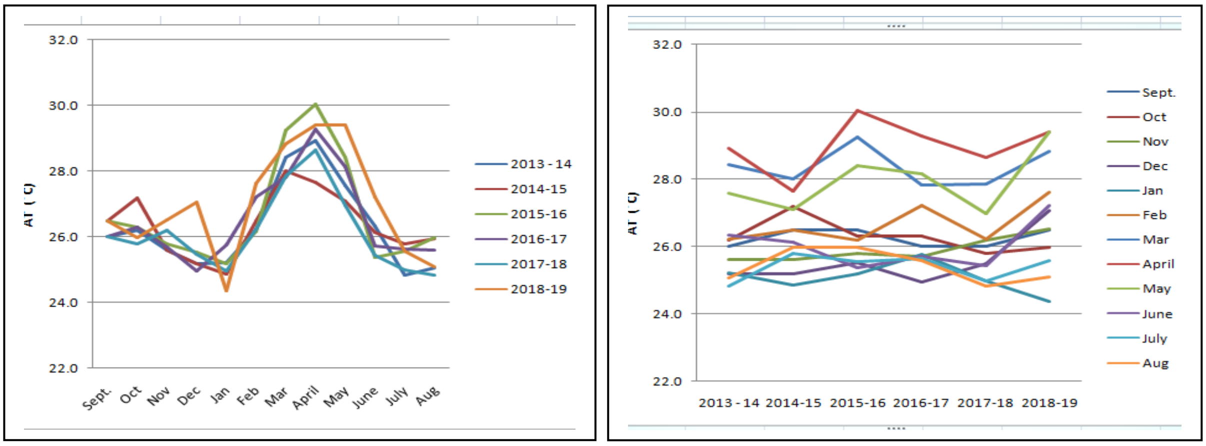
(a) (b)

Fig. 5.5. Pattern of ambient temperature across months (a) and six years (b)

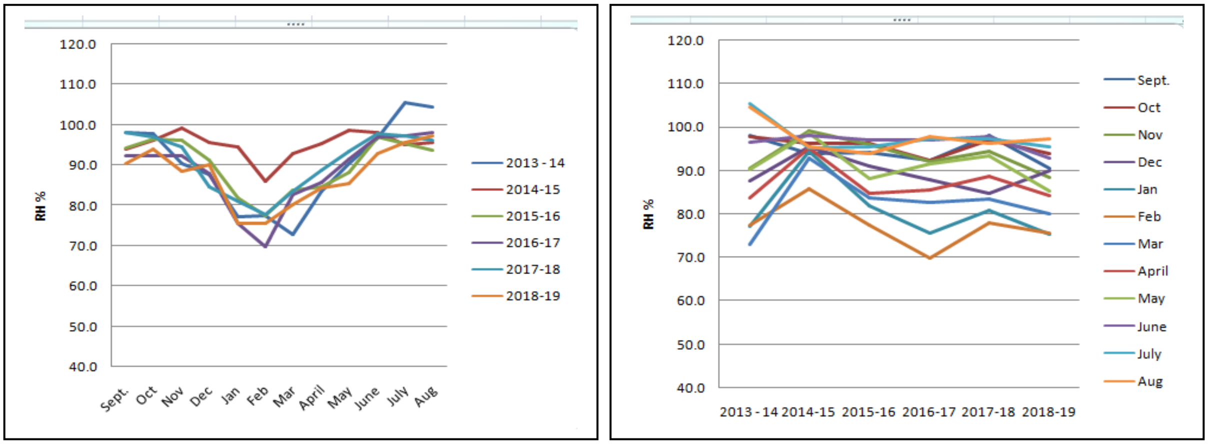
(a) (b)

Fig. 5.6.Pattern of relative humidity across months (a) and six years (b)

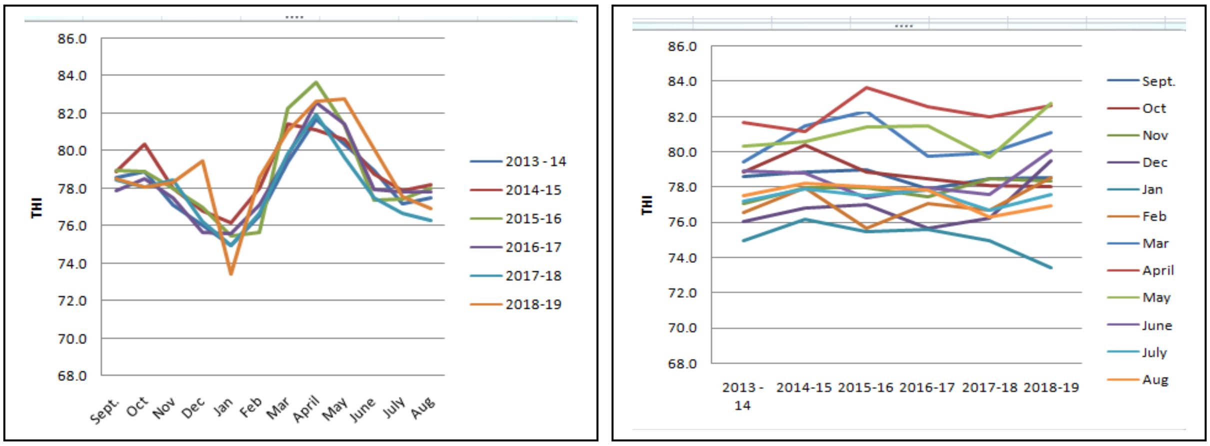
(a) (b)

Fig. 5.7.Pattern of THI across months (a) and six years (b)

yearly pattern shown in Fig. 5.5 to 5.7.However, the stock and breeding related parameters varied significantly between years as shown in Table 4.9. Many of the fertility parameters also showed significant variation between years (Appendix 1) and the same can be attributed to the variations in management strategies between years.

There was significant association of fertility parameters with some of weather parameters across years as shown in Table 4.10.Even though THI was not having significant correlation with any of the study parameters, different variants of AbT and RH showed significant correlation with few of the fertility parameters. Also the strength of correlation was more with RH variants than AbT, indicating lesser influence of TS and more of incidental association. Likewise, the inter-relationship among various fertility parameters shown in Table 4.11 varied with respect to strength of correlation as well as the nature of relationship, lacking uniformity of the pattern. Thus, the correlations appeared to be due to inter-dependence of fertility related parameters each other rather than influence of TS.

5.1.5.3. Herd Fertility Status

Even though mean fertility indices of the herd for the six years showed no significant seasonal variation, indicating almost uniform reproductive performance irrespective of the seasons and prevailing climate, the overall reproductive performance with respect to each of the indices was very low based on expected standards (Kutty, 2004) or targets fixed as shown in Table 5.1.

Table 5.1. Herd fertility indices of the six years compared with expected figures

Fertility index	Reproductive performance		
	Achieved	Expected	Achievement (%)
Age at first calving (months)	34.5±1.6**	24	70
Onset of PP oestrus (days)	71.8±5.7**	40	56
Service period (days)	110.9±8.1**	60	54
Inter-oestrus interval (days)	39.4±1.2**	21	53
AI per conception (Number)	2.7±0.1**	2	74
Calving to conc. Interval(days)	224.7±16.2**	80	36
Inter calving interval(days)	479.8±17.1**	365	75
Conception rate (%)	36.6 ± 2.9**	50	37

** significant(P<0.001)

The achieved performance was not more than 75 per cent of the expected level for any of the indices, between 50 – 75 per cent for six indices and less than 50 per cent for two indices such as calving to conception interval and conception rate. Thus, it can be inferred that the dairy cattle herd under the study have reduced reproductive performance and one of the reasons appeared to be TS since the overall management was near optimum. This is in consonance with many earlier reports of low reproductive performance of animals exposed to TS (Mapham and Vorster, 2013; De-Rensis *et al.,* 2017; Krishnan *et al.,* 2017).

Above findings imply that fertility parameters compared in the study were not much affected by weather parameters during the study period, even though the THI values were beyond the limit of thermal comfort for dairy cattle throughout the year. Possible explanation for the lack of variation between seasons can be the adaptability of these animals to the prevailing adverse climate through continuous rearing at the same place and causing passive selection over the years. This is in agreement with the earlier reports that crossbred cattle get adapted to local climate upon continuous exposure (Hansen, 2009; El-Tarabany and El-Bayoumi, 2015;Macias-Cruz *et al.*, 2016; Archana *et al.*, 2017).

5.2. PROSPECTIVE STUDY

Started with preliminary steps in July 2018 such as; selection of animals, setting up of data collection devices, designing and fabricating movable trevis and trials on USG in the study setting. Actual prospective data collection was started from 1st Sep 2018 and continued for a period of one year until 31st August 2019. Follow up activities were continued for two more months such as laboratory works for completion of blood sample testing, pregnancy verification of animals bred, data entry and statistical analysis.

5.2.1. Weather parameters

The annual trends of both macro and micro climatic recordings of AbT and RH are shown in Fig 5.8 and 5.9 respectively. The month of lowest AbT and RH from both micro and macro climatic recordings happened to be January attributable to a major drop in AbTconsequent to the cessation of the wind prevalent in the region. Except for such a fall, the AbT was showing an inverse relationship with RH (p-value = 0.001) across the months. The month of highest AbT was found to be different for macro and micro recordings caused by a drop in temperature inside the shed, while outside temperature was still on the rise. This was

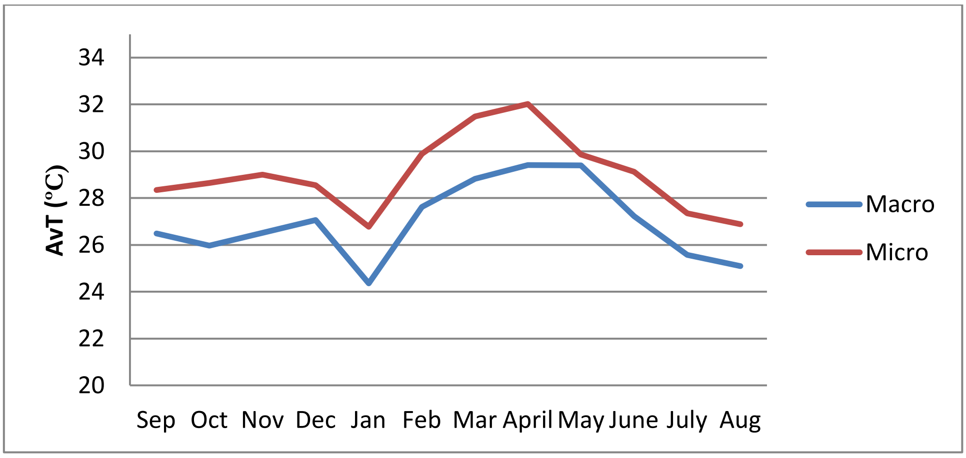

Fig. 5.8. Macro and micro climatic ambient temperature across months

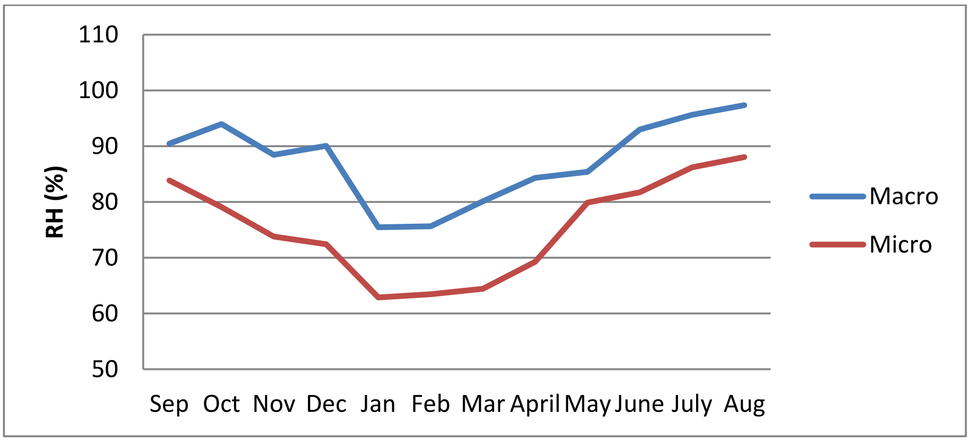

Fig. 5.9. Macro and micro climatic relative humidity across months

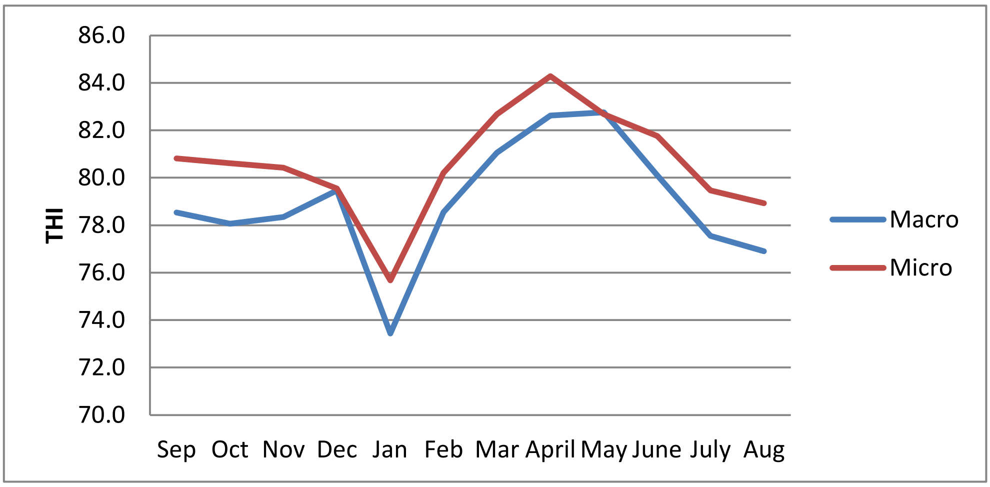

Fig. 5.10.THI based on macro and micro climatic recordings across months

found to be associated with an elevation of micro climatic RH as well, attributable to some management alterations such as spraying water on to the cows during hot hours.

As shown in Table 4.12. macroclimatic recording of AbT was lower (the difference being significant - $P<0.05$) than the same from microclimatic source during all the months (Fig. 5.8), and the same appears to be due to better cooling of air outside the barn especially during the night. In addition, breathing out air from the animals and thermal radiation from the roof adds to the elevation of AbT inside the shed. At the same time RH showed opposite pattern ($P<0.05$) with a higher level outside the shed than inside (Fig. 5.9) and the same appears to be the effect of rain and dew formation during the night and morning hours. In earlier studies at other parts of the state, AbT and RH outside and inside the sheds showed opposite pattern compared to the present study (Prasad, 2014; Harikumar, 2017), which may be due to the difference in the study settings and the type or positioning of the devices used.

The macro - micro differences of both AbT and RH were less during May to September as shown in Table 4.12. This indicates that both inside and outside weather becomes closer during this period and may be due to better cooling of the air by the rain together with increased air movements lowering the inside temperature as well.

5.2.1.1. Temperature Humidity Index

Temperature Humidity Indices from micro and macro recordings across months are compared in Fig. 5.10. It is evident that THI has the same pattern as AbT (Fig 5.8) since THI gives more emphasis on temperature as the causative factor of TS. The pattern of RH is opposite to that of THI due to the inverse relationship between RH and AbT.

As it is evident from Table 4.13, the values of THI lies between that of AbT and RH since both these variables pull the THI value in opposite direction. Seasonal variations of AbT and RH from both micro and macro recordings and THI from micro recording were significant between seasons. However, in spite of significant variations of AbT and RH, variation of THI from macroclimatic recording was not significant. Thus, microclimatic THI is more important not only due to significant variation between the seasons, but also due to its direct influence on the animal.

Comparison of THI (calculated from daily averages of AbT and RH) with weather parameters (Table 4.14) showed highly significant ($P<0.001$) correlation with AvT and

MnT, lesser association ($P<0.05$) with AvRH and no significant correlation with MxT, MxRH and MnRH. The correlation of THI with AvT and AvRH shows their proportionate contribution to THI. Whereas highly significant positive correlation of MnTwith THI shows that MnT forms a major indicator of TS compared to MxT.

Elevation of MnT affects thermal comfort of dairy animals reducing the chances of passive heat dissipation. Moreover, elevation of MnT occurs during the period of maximum day length as mentioned earlier so that MxT and duration of exposure to adverse climate increases simultaneous with MnT. Further,MxT is often associated with a decrease of RH especially in Kerala climate and increases the chances of evaporative cooling, while MnT is associated with higher RH and less evaporation. Thus elevation of MnT is one of the major determinants of TS affecting heat dissipation potential as reported by Sonmez *et al.* (2005).

Between half years of short and long days as well as rainy and non-rainy periods, RH was significantly high during rainy months (Table 4.15), whereas AbT and THI did not show significant variation between the half years. While AbTshows significant elevation during the long day period in other parts of the country, early occurrence of monsoon in the state (Kumar, 2013; Rao, 2013) suppresses the temperature so that AbT is not significantly high in both the half years.

In most tropical regions, high THI causing moderate to severe stress and low THI within the comfort level of animals occur in two separate half years. However, consequent to early occurrence of monsoon in Kerala, high RH and low temperature causes THI reduction during JJA. During the other seasons, even though RH becomes slightly lesser,AbTdo not fall very much so that moderately high THI is maintained throughout the year and without causing significant variation between the seasons. This makes the Kerala climate very unique, but very unfavorable for high producing animals since the THI remains always high at a level to cause mild to moderate stress throughout the year.

5.2.1.2. Other Climatic Stress Factors

Monthly trend of minimum and maximum of AbT and RH are shown in Fig. 5.11 and 5.12, respectively. While the pattern of AbT is more or less same as that of retrospective study, RH pattern was slightly different, attributable to the difference in rain fall between the years. As shown in Table 4.16 the difference between maximum and minimum of AbT and RH across months increased from September onwards until the maximum during January to

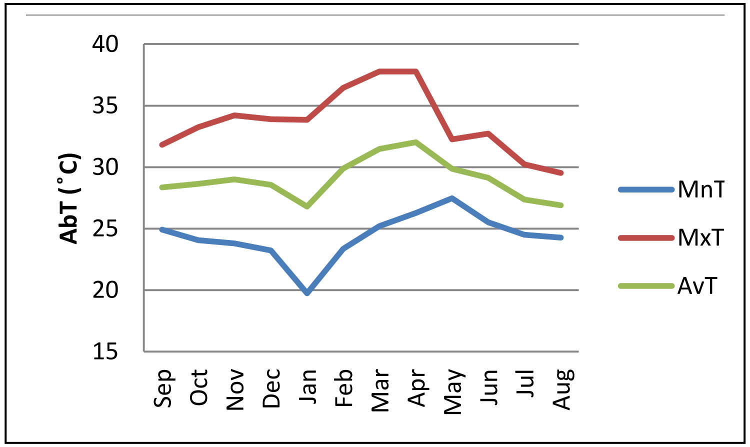

Fig. 5.11.Monthwise trend of ambient temperature

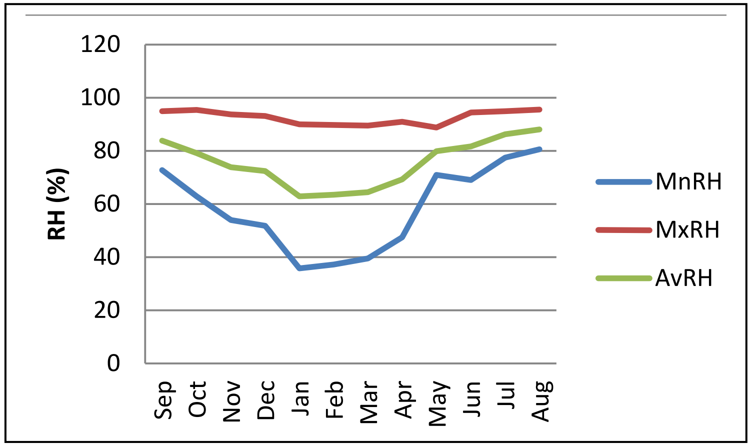

Fig. 5.12. Monthwise trend of relative humidity

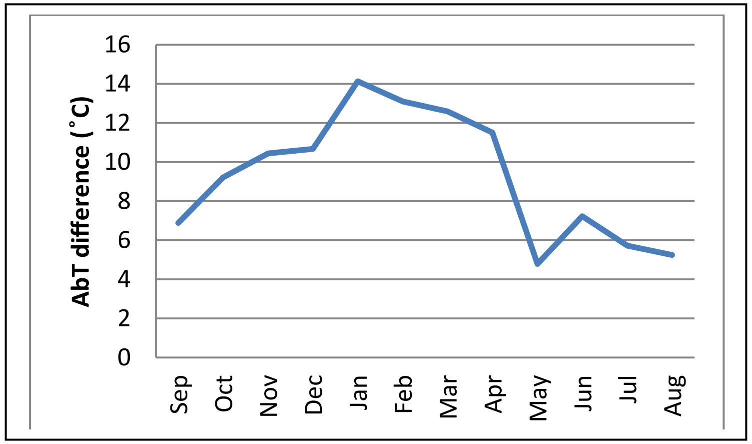

Fig. 5.13.Difference of maximum and minimum ambient temperatures across the months

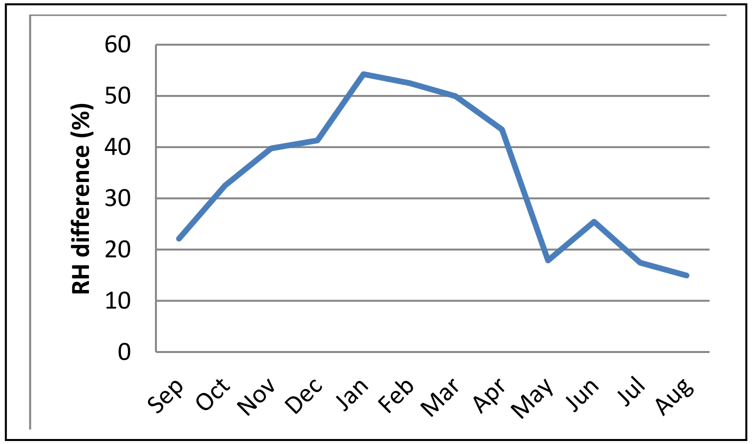

Fig. 5.14. Difference of maximum and minimum relative humidity across the months

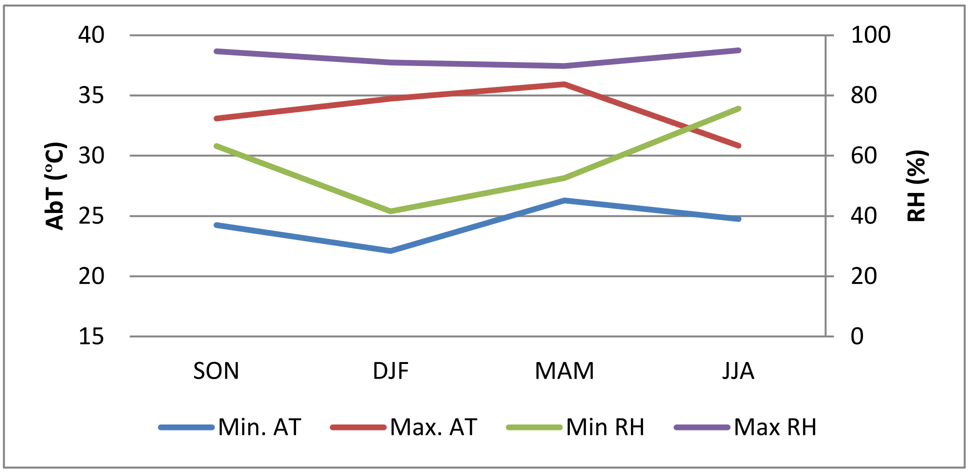

Fig. 5.15 Quarterly trend of max - min values of AbT and RH

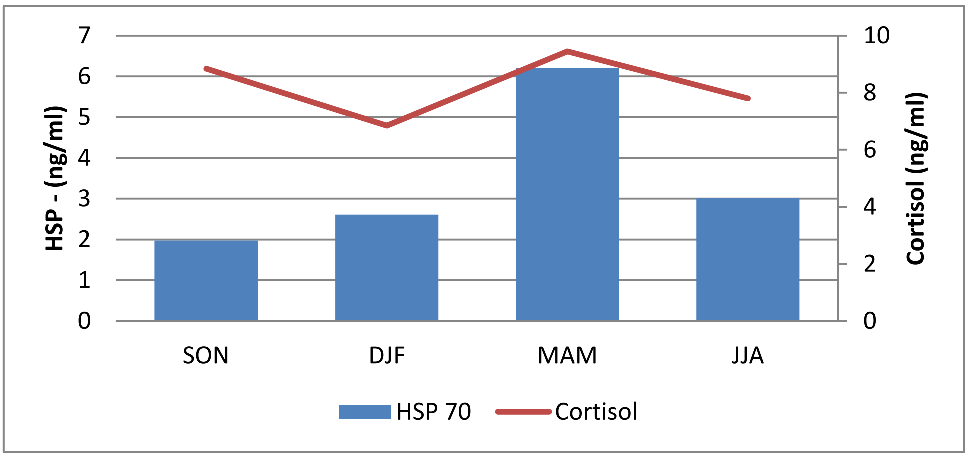

Fig. 5.16. HSP and Cortisol across seasons

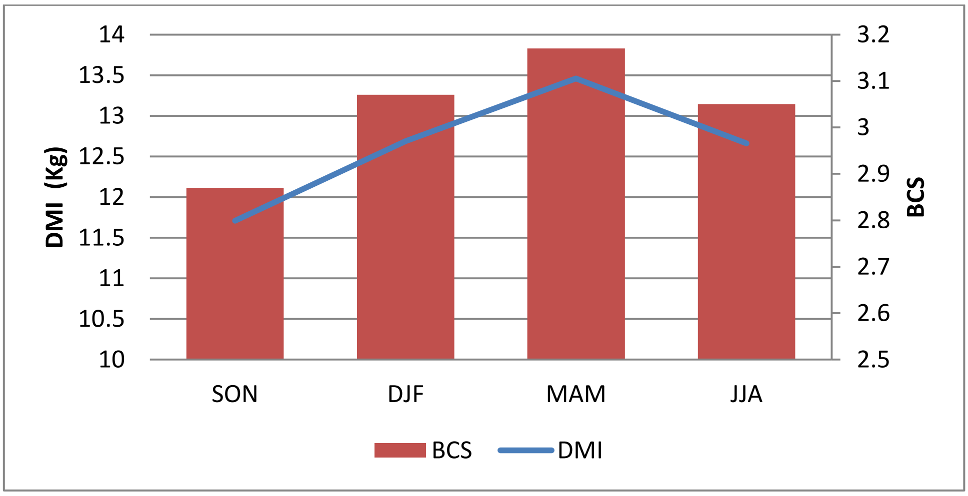

Fig. 5.17. Comparison of BCS and Dry matter intake

March and decreased there after again to the lowest in August. This is attributable to the longer night hours causing better cooling, lack of rain causing more warmth during day time and the inverse relationship of AbT and RH.

The variation patterns of the differences between maximum and minimum values of AbT and RH across the months are shown in Fig 5.13 and 5.14, respectively. Strikingly, both the graphs show similar pattern of variation, even though AbT and RH maintains an inverse relationship with each other. This is attributable to the difference in the extent of influence of rain fall on MxT and MnRH as evidenced in the Figure 5.15, showing simultaneous plotting of the quarterly means of maximum and minimum AbT and RH. It appears that the association of MxT with MnRH is very sensitive than with MxRH. While maintaining inverse relationship with both, even small increase of MxT causes major decrease in MnRH, while change of MxRH is very less. Thus the difference between MxRH and MnRH increases even for any small increase of MxT and vice versa.

Thus along with increase of daily averages of AbT and RH, extend of variation between MxT and MnT and levels of elevation of MnT are two important factors deciding TS to the animals concurring the findings of Maurya *et al.* (2005) and Prasad *et al.* (2013). Quarterly means of MnT, MnRH, MxRH and difference between MnRH and MxRH varied significantly as shown in Table 4.17. However, the distribution of highest and lowest figures of each of these variables did not show any similarity across seasons. Hence, it can be inferred that highest variation between maximum and minimum of AbT (12.62ºC) and RH (49.34 %) during DJF predisposes to increased TS even during post monsoon, though THI is comparatively low during this period.

5.2.2. Seasonal Pattern of Thermal Stress

Even though well defined seasons comparable to temperate region is lacking in the tropical climate, seasonal pattern of reproductive performance has been well established among farm animals (Kutty, 2005; Sonmez *et al.,* 2005). Various factors have been reported as determinants of the seasonal variations (Bouhroum *et al.,* 2014), major one being TS (Wolfenson *et al.,* 2000; Collier *et al.,* 2017)influenced by AbTand RH (Kutty, 2005; De-Rensis, *et al.,* 2017).

Photoperiodicity also forms major regulator of seasonality and TS. Besides direct influence through Pineal gland secretion, photoperiodicity influences reproduction in animals indiretly by regulating AbT and RH. Pattern of day length variation in Kerala reported by Kutty (2013) is illustrated in Fig. 5.18. Also the patterns of monthly variations of AbT, RH and THI are also compared with day length in Fig. 5.19 to 5.21.

It is evident from the graphs that summer season in Kerala does not correspond to the period of maximum day length and such a difference from other parts of the country is attributable to the monsoon occurring from June onwards. Since RH is regulated mainly by rainfall, there is no prominent association of RH with day length.Even though AbT and THI were increasing from January onwards with increasing day length as in other parts of the country, early onset of monsoon causes a sudden drop in AbT and THI, which is responsible for the peculiar Kerala climate as mentioned earlier (Rao, 2013).

5.2.3. Influence of Thermal Stress on Animals

5.2.3.1. Stress Indicators

Among the biological factors, HSP 70 and cortisol are considered as direct indicators for physiological stress. While cortisol represents stress out of various reasons, HSP 70 is considered more specific for TS (Rajoriya *et al.,* 2014). Mean values of serum HSP 70 showed highly significant variation ($P<0.001$) between months, seasons and half years of long / short days and also rainy / non-rainy seasons (Table 4.18 and 4. 44). This indicates that HSP 70 is more sensitive and the animals were subjected to different levels of TS during each of these periods of comparison.

All the major climatic determinants of TS such as MnT, MnRH, AvT, AvRH and THI showed highly significant correlation ($P<0.001$) with HSP 70 (Table 4.19), indicating HSP 70 as a better biological marker for TS. Min *et al.* (2015) also recommended HSP 70 as a potential biomarker to supplement THI and to evaluate moderate TS in dairy cows.

Gaughan *et al.* (2013), Rajoriya *et al.* (2014) and Zarina (2016) also reported significant up-regulation of HSP level with AbT. However, cortisol level did not show significant correlation (Table 4.19) with any of the weather parameters across seasons, in agreement with the report of Torres-Junior *et al.*(2008), indicating involvement of non-TS factors as well in regulating cortisol levels. Some studies also reported strong relationship of HSP 70 with day length (Gaughan, *et al.*, 2013) and RH (Rajoriya *et al.*,2014).

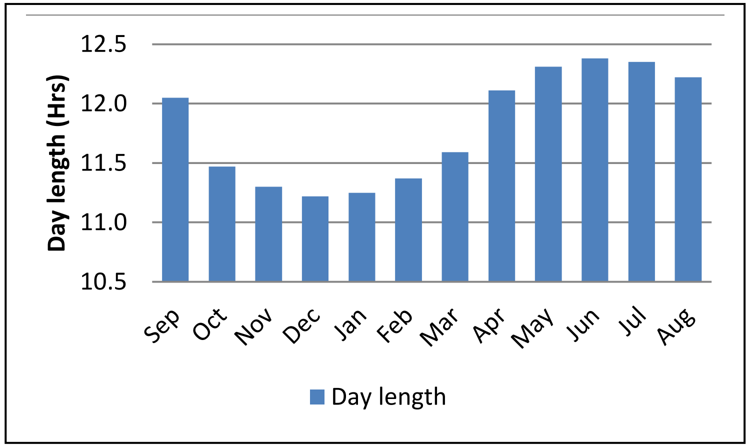

Fig. 5.18.Pattern of day length across months

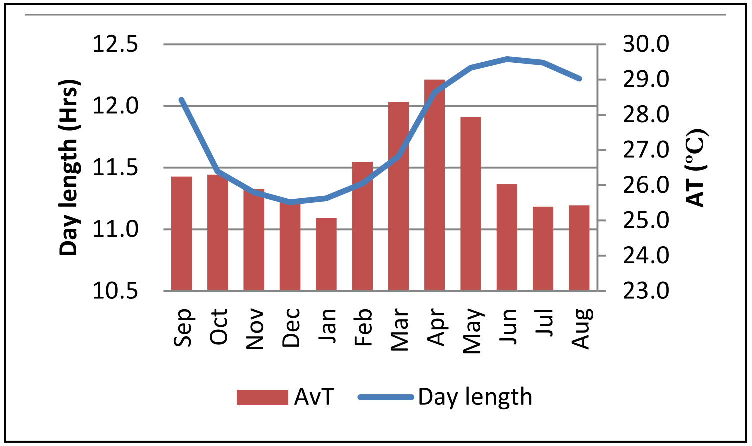

Fig. 5.19. Day length and Av. temperature across months

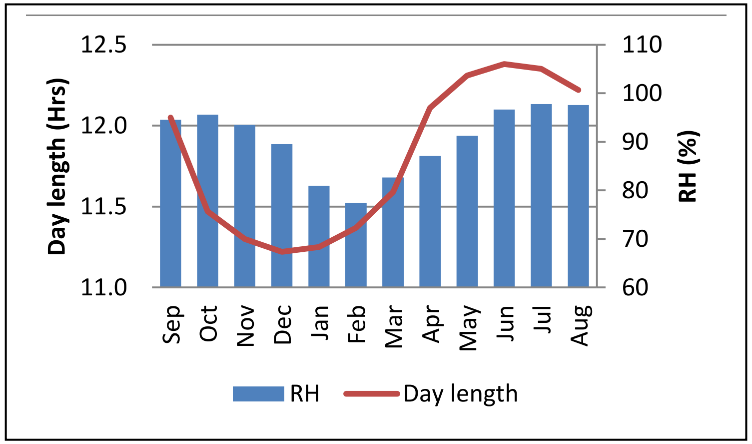

Fig. 5.20.Day length and RH across months

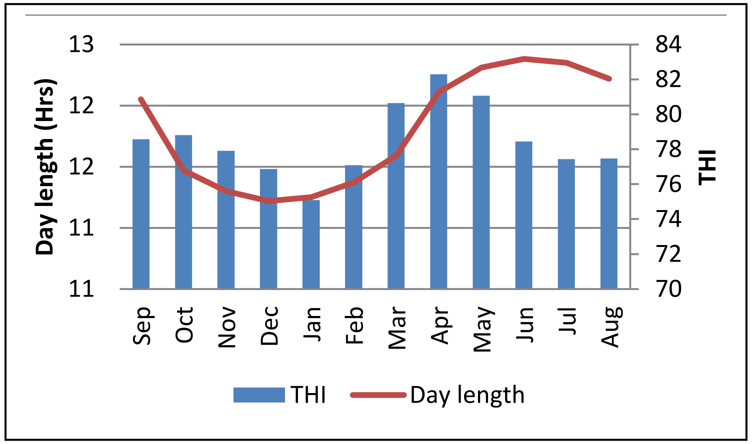

Fig. 5.21. Day length and THI compared across months

HSP 70 and cortisol values varied significantly between seasons (P<0.01) which agrees with earlier reports of Mishra and Palai (2014) and Rajoriya *et al.*(2014). Quarterly averages of HSP 70 and cortisol were highest during MAM – the period of highest AbT and THI (Fig. 5.16), which is in agreement with the report of Rajoriya *et al.* (2014). However, the periods of lowest mean values were different for both HSP and cortisol being SON and DJF, respectively (Table 4.18). Both these periods were having almost same and lowest AbT (28.66 ºC versus 28.40 ºC) across seasons.

It is evident that the pattern of HSP 70 variation is more similar to that of AbT (also evidenced by stronger correlation with AbT than other weather parameter), while cortisol variation is more identical with the pattern of THI indicating more influence of THI on cortisol levels than HSP. Significant variation of the HSP 70 and cortisol coinciding the periods of high AbT and THI, with significant positive correlation indicates that the study animals are affected by TS. Hence, these biological factors especially HSP 70 levels were compared with each of the other study parameters and between seasons to understand theinfluence of TS and involvement of climatic factors on the welfare and reproductive performance of the animals.

5.2.4. Animals Selected for the Study

Each animal enrolled during the early PP (7-10 days of calving) was maintained under the study (Gr. 1 and 2) for 3 to 4.5 months depending upon the availability of replacement. A total of 82 animals were included under the study on a knock out basis maintaining at least 22 under the study all the time. Further, out of the 22 cows, 8 were used for detailed study every month during their Day 28 to Day 91 PP.

5.2.5. Routine Management

Selected animals were housed together during the study period to avoid possible variation of management and to minimize any stress out of shifting between the sheds. No cooling system was provided during any of the months except fans operated and sporadic spraying of water on to the animals during hot hours of the summer months as part of the routine management.

5.2.6. General Parameters

Details of daily feeding, milk yield and BCS at weekly intervals were recorded regularly for a period of one year

5.2.6.1. Effect of Thermal Stress onDry Matter Intake

Concentrate intake was assessed based on the feed offered to each animal on the basis of feed computation revised every week considering the requirements for maintenance, growth and lactation. Feed was offered in individual feeding units at two timings such a way that enabled consumption of the total quantity avoiding wastage and cross feeding of nearby animals so that feed offered represented the total intake of concentrate.

Increased DMI intake is usually accompanied by subsequent increase in the metabolic rate and elevation of BT so that there occurs a reduction of feed intake very often especially during summer months (Abdalla *et al.,* 2017). However, in the present study, DMI was significantly high ($P<0.05$) during the summer, lowest in SON and more or less similar in DJF and JJA (Table 4.20). This can be attributed to mild or moderate level of TS suffered by the animals, which has been estimated to cause an increase in the maintenance requirement by 7 to 25 per cent (Allen *et al.,* 2009). However, severe TS have been found to cause lactating dairy cows to enter a period of negative energy balance (Settivari *et al.,* 2007; Rhoads *et al.,* 2009).

Increased DMI during the period of maximum TS coincide with the increased milk production (non-significant correlation) and BCS (correlation significant at 5 % level). Gaughan *et al.* (2013) also reported significant correlation of feed intake with BCS as well as HSP 70. Sonmez *et al.* (2005) found that TS did not alter DMI throughout the year. Similarly Gomez *et al.* (2018) reported increased feed intake and better conception in high producing cows even during adverse weather conditions. However, findings of the present study is in contrary to many earlier reports that as AbTincreases, DMI decreases (Settivari *et al.,* 2007;Torres-Junior*et al.,* 2008; Rhoads *et al.,* 2009) and this appears to occur as a biological measure to reduce metabolic heat (Torres-Junior*et al.,* 2008).

DMI did not show significant correlation with weather parameters other than MxRH in this study (Table 4. 21). This may be due to the temperature hike to the extent for causing only moderate stress during MAM and mild stress during rest of the seasons indicated by THI values. Whereas, significant negative correlation ($P<0.01$) with MxRH can be due to coincidence of rainy season, wherein the fodder intake is reduced. Contrary to the present study findings, decrease in DMI to the extent of 23 per cent has been reported in heifers subjected to high AbT by Ronchi *et al.* (2001).

5.2.6.2. Influence of TS on Milk Yield

Comparison of milk yield across stages of lactation showed higher yield during first two months after calving. Across seasons, maximum yield was during MAM, and correspondingly, the highest yield was achieved when these two periods overlap. The differences in milk yield between either of these periods were not significant separately, even though their combined influence showed significant variation across lactation stages with the highest and lowest in first and third months, respectively (Table 4.22). Milk productiondeclined rapidly from the third month as reported by Petrovska and Jonkus (2014), whereas in the present study, those animals attained 61-90 days and above 90 days of their lactation during MAM showed less reduction of yield compared to others.

Reduction in milk yield is primarily caused by reduced feed intake. However, Rhoads *et al.* (2009) reported that factors other than reduced feed intake are responsible for 64% of the overall milk loss during the periods of TS. The animals calved in the months of January to April contributes more milk during MAM being in their first two months of lactation and are fed accordingly leading to more concentrate intake. As the result, increased milk production and better BCS are maintained during MAM, as reported by Gomez *et al.* (2018). More standing time and feeding during night hours in summer has also been reported as behavioural alteration to meet the increased nutrient demand that leads to increased feed intake of summer(Allen *et al.*, 2009).

The study finding of increased milk yield is contrary to the normal expectation of milk yield reduction with elevation of AbT(Kadzere *et al.*, 2002; Kristyna *et al.*, 2017; Guo *et al.*, 2018). Such a phenomenon can be attributed to adaptation of these animals since they are exposed to TS of mild to moderate degree throughout the year over many years. Metabolic and physical alterations favouring adaptation might have made them capable of producing more milk and eat more even during the summer as opined by Prasad (2014). Rhoads *et al.* (2009) reported that metabolic adaptations caused by TS prevent some of the glucose-sparing mechanisms that normally avoid severe reductions in milk yield during periods of inadequate nutrient intake.

5.2.6.3. Influence of TS on Body Condition Score

The BCS was significantly low (2.87) during SON compared to other three seasons (Table 4.23) and is comparable with the low feed intake during the season. The lesser average BCS of around 2.6 has been reported at the time of AI in high-producing lactating dairy cows by Gomez *et al.* (2018). Reduced feed intake consequent to TS forms the major reason for reduced body condition (De-Rensis*et al.,* 2017; Guo *et al.,* 2018). Comparison of BCS and DMI across seasons are shown in Fig. 5. 17. The patterns of variation of both these parameters were similar across seasons as reported by Das *et al.* (2016).

Increased milk production greatly contributes to loss of body condition. Hence, BCS sorted based on the stage of lactation did not vary significantly between seasons (Table 4.24) as against significantly low value of raw BCS during SON. This implies that whatever difference in BCS noticed between seasons was attributable to the effect of lactation.

Comparison of BCS and milk yield (both raw and corrected for stage of lactation) with weather parameters showed no significant correlation with any of the weather parameters except the association of maximum values of AbT and RH with lactation stage adjusted figures of BCS and milk yield, respectively.This association appears irrelevant as a causation of TSsince maximum values of AbT and RH do not occur simultaneously and for the same reason, THI was not having any significant correlation with BCS and milk yield.

Among the biological stress indicators, HSP 70 had significant positive correlation ($P<0.05$) with level of feeding as well as BCS, while cortisol was positively associated ($P<0.05$) with BCS alone (Table 4.21). Gaughan, *et al.* (2013) reported a highly significant correlation of BCS and HSP 70. A positive association of BCS with fertility was reported by Gomez *et al.* (2018). Loss of body fat and BCS has been found to be associated with delayed postpartum ovulation in dairy cows (Morales *et al.,* 2018).

This means that increased feeding and BCS during summer coexist with stress in these animals and the variation in milk production (non-significant) appeared to be not affected by stress. These findings are contrary to earlier reports and indicate adaptation of the study animals with climate prevalent in the locality as opined by Rhoads *et al.* (2009).

5.2.7. Reproductive Management

Reproductive management parameters such as number of oestrus detected, intensity of oestrus and details of AI done were studied in both the groups of animals. Even though detection of oestrus was carried out by visual observation at 6-8 hour interval during day and night, verification of the breeding history, confirmation of oestrus by gynaecological examination and AI in required cases was performed only during day time for operational convenience.

5.2.7.1. Detection of Oestrus

Among the 82 animals under study, 63 oestrus periods were detected during the period of two to three months starting from 28 days PP. Total number of oestrus detected was less (Table 4.27) compared to overall oestrus detection rate of the herd obtained from retrospective data and is attributed to the focus only on early postpartum animals and lesser occurrence of oestrus during the transition period (Leroy *et al.,* 2008).

Between the groups, number of oestrus detected was more in Gr.2 than Gr.1 being 52 and 11 respectively. Higher proportion of oestrus detected among Gr. 2 animals can be attributed to close monitoring with weekly gynaecological examination and USG, which is believed to enhance the occurrence of oestrus as well as chances of detection (Opsomer *et al.,* 1996; Orihuela 2000; Maurya *et al.,* 2017).

Proportion of animals detected in oestrus out of the total observed varied significantly between seasons (Chi square p-value<0.01), with the highest during DJF and lowest in JJA. More oestrus during DJF can be due to favourable weather with lowest MnT, MnRH and THI, whereas lowest number of oestrus during JJA may be due to delayed effect of TS after the summer. Earlier studies also has indicated that TS affects the length of oestrous cycle causing more of short cycles, irregular cycles and even loss of cyclical activity (Torres-Junior *et al.*, 2008).

The pattern of variation between seasons in the number of oestrus detected and animals involved was not appeared to have any association with the variation of any of the physical, biological and weather parameters studied. Moderate elevation of AbThas been reported to affect the behavioural and physical manifestations of the oestrus so that as many as 80 per cent of oestrus goes undetected (Roelofs *et al.,* 2010; Das *et al.,* 2016). Behavioural manifestations of oestrus are already very less in the herd and lack of any obvious relationship with the study parameters can be attributed to the involvement of some other factors as well in the regulation of oestrus occurrence and detection of oestrus.

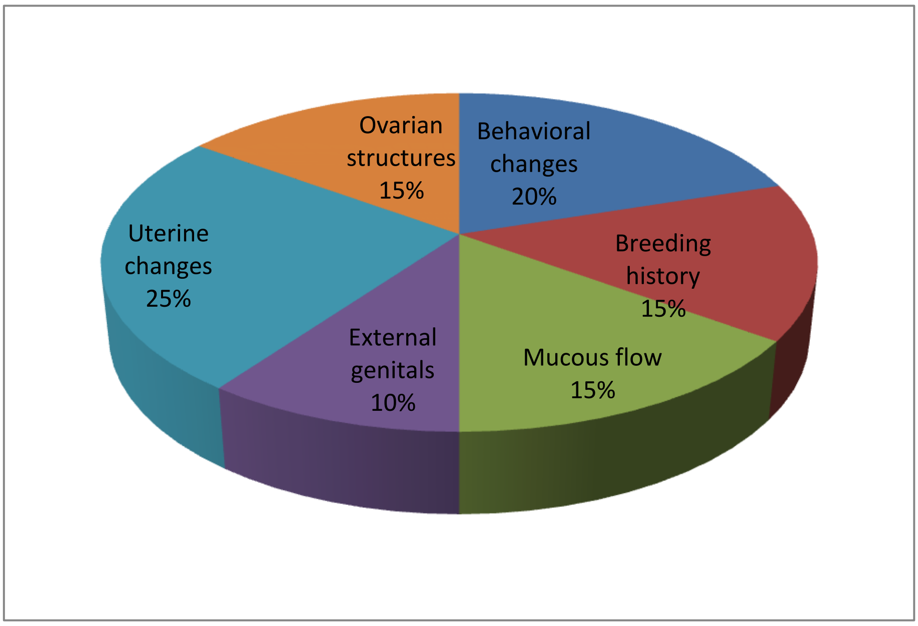

Fig. 5.22. Weightage for oestrus signs and associated changes in the intensity scoring

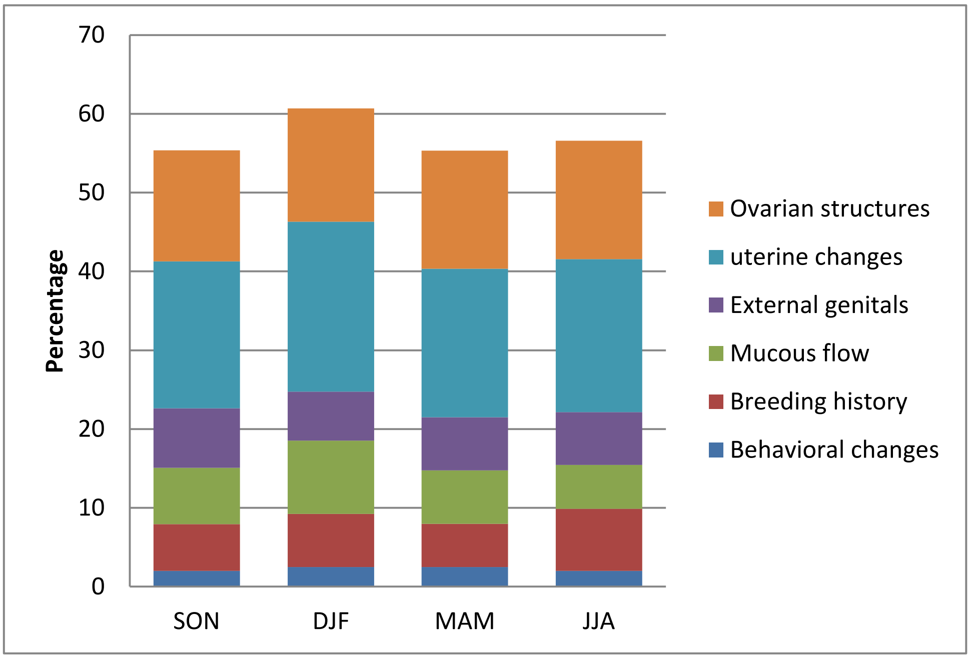

Fig. 5.23. Oestrus intensity scores compared between the seasons

5.2.7.2. Intensity of Oestrus

Different oestrus signs checked and maximum scores allotted for each in order to assess the intensity of oestrus on the basis of history, visual observation of external signs and clinico-gynaecological examination are compared in Fig. 5. 22.

Comparison of the oestrus intensity assessed based on the score sheet (Fig. 5. 23) did not show significant variation between seasons as shown in Table 4. 28. Weakening of oestrus signs was reported during summer months and attributed to the alterations of endocrine mechanisms (Bolocan, 2009). Inhibition of oestradiol secretion by the growing follicles has been mentioned to cause lesser intensity of oestrus signs during summer by Hansen (2015) and Sonmez *et al.* (2005).

Even though there were variations between animals in the nature of signs, in agreement with Bolocan (2009) and Macias-Cruz *et al.* (2016), and the scores were assigned for individual signs with due weightage, overall score was statistically non-significant between seasons. Paul (2016) also reported more or less similar oestrus intensity irrespective of THI and seasons. This may be due to lesser variations of secondary oestrus signs between seasons contrary to the earlier reports of lack of visible signs in summer months alone (Lopez-Gatius *et al.*, 2008; Roelofs *et al.*, 2010; Macias-Cruz *et al.*, 2016).

The intensity scores were slightly better during DJF, may be due to congenial climate, but lesser and more or less same level during other seasons including summer. Comparatively more contribution to the total intensity score was observed from uterine changes across all seasons and especially during DJF. However, contribution of the score by behavioural signs was the leastas shown in the Figure 5.23 as well as Table 4.28 and is attributable to the progressive reduction of behavioural signs already reported in the herd (Kutty, 2005; Nasir and Kutty, 2004). .

Comparison of oestrus intensity score across seasons with other study parameters showed an inverse relationship with cortisol, THI and MnT. This association of cortisol indicates that besides elevation of AbT, intensity of oestrus signs are affected by other stress factors affecting the comfort of living. Schuller *et al.*, (2017) also reported significant reduction in the intensity of oestrus signs with increased THI. However, other than stress and season, various management and social factors has also been found to influence the expression of oestrus behaviour and oestrus detection rates (Roelofs *et al.,* 2010; El-Tarabany and El-Bayoumi, 2015).

In the present study, the intensity scores were more or less same during all the seasons in agreement with Paul (2016) and there is more similarity of the seasonal pattern with that of cortisol and not with that of HSP 70. Hence, it can be inferred that more than TS, it is the general stress that influences the intensity of oestrus signs causing weakening of behavioural manifestations in crossbred cows which is in consonance with the earlier reports by Nasir and Kutty (2004).

5.2.7.3. Artificial Insemination

Out of 38 inseminations done, only 5 were in Gr. 1 and 33 were in Gr.2. (Table 4.29), which correspond to thedifference in the number of oestrus detected in each group (11 and 63 respectively). Double AI during the oestrus was more in Gr. 2 (57.58 %) than in Gr. 1 (40.00 %). Persistence of follicle found during USG of ovaries on second day of oestrus in Gr. 2 cows, attributable to delayed ovulationformed the major reason for high proportion of DAI. Even though the proportion of DAI is more in Gr. 1 as well, total number of AI is very less to interpret. Proportion of AI among cows in oestrus was almost the same in both groups. However, 19 out of 33 AI in Gr. 2 and 4 out of 5 AI in Gr. 1 did not returned to oestrus. More non-return in Gr.1 is attributable to poor oestrus detection and was clarified subsequently by detection of only one pregnancy among Gr. 1.

Monthly averages of oestrus detected and AI done (Table 4.30) were more in DJF and lowest in MAM, even though the total number of oestrus detected and AI done were less and variations between seasons were non-significant. Number of AI done did not show significant association with any of the study parameters(Table 4.31 and 4.32) and is attributed again to low numbers of AI done, being the study of early PP period.

5.2.8. Detailed Study

5.2.8.1. Cows Selected for Detailed Study

Numbers of cows under the detailed study during each of the months (Gr. 2) were eight, of which four were replaced every month with new PP cows randomly selected from the 22 under the study. Detailed study period extended from 28 to 91 days PP.

5.2.8.2. Respiration Rate and Rectal Temperature

Respiration rate and RT recorded at weekly intervals from the eight animals each during DJF and MAM showed highly significant ($P<0.01$) variation between months and seasons(Table 4.33) concurring the report of De-Souza *et al.* (2016). Elevation of RT occurs whenever the heat production increases due to increased metabolism and /or affection of heat dissipation (Mishra and Palai, 2014). Whenever AbT increases passive heat dissipation is reduced and RT starts to increase, unless active heat dissipation mechanisms are initiated (Macias-Cruz *et al.,* 2016). Since dairy animals are more comfortable at low temperature (below 20 ºC) elevation of AbT beyond thermo neutral zone leads to rise of BT and causes activation of breathing rate as the immediate response (Rashamol *et al.,* 2018).

Between the two seasons, there was marked variation in AbT and THI values, causing significant variation of RT and RR between months, seasons and even between eachtimes of recording (Table 4.34 and 4.35), indicating marked diurnal variation as reported by Guo *et al.* (2018) and Rashamol *et al.* (2018). Marked elevation of RT has been reported to have associated with TS by Torres-Junior *et al.* (2008). Both RT and RR had almost similar pattern of variation as shown in Fig. 5.24 and 5.25, with the rates decreasing from December to the lowest in January and continuously increases thereafter(De-Souza *et al.,* 2016). The highest RR was attained during April and started to decline thereafter, while RT started to decline little more later reaching the highest only in May. This difference in the monthly pattern of RR and RT is explained below with similar variation of daily pattern as well.

Between seasons, there was not much difference between the patterns of RR and RT, and marked elevation of both occurred during MAM as reported by Morris *et al.* (2011), De-Souza *et al.* (2016) andMaibam *et al.* (2017). Between the two parameters, the pattern of daily variation showed somewhat similar pattern as that of monthly variation such that RR was highest at 2 PM and there after declined (Fig. 5.26), where as RT continued to increase upto 4.30 pm and then only started to decrease (Fig. 5.27). Thus, why the RR decreased even while RT was increasing needs more explanation?

The AbT starts to decline after 2.30 pm so that the chance of heat dissipation from the body starts to increase. Since active breathing itself causes generation of more metabolic heat, active effort for breathing is suppressed following the onset of AbT reduction.

Thismight be the reason for reduced RR well before the onset of RT to decrease. Consequent to

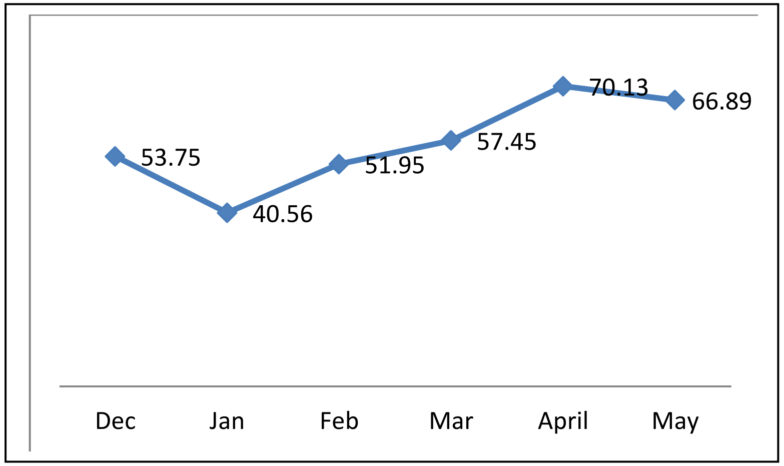

Fig. 5.24. Monthly averages of respiration rate of eight cows

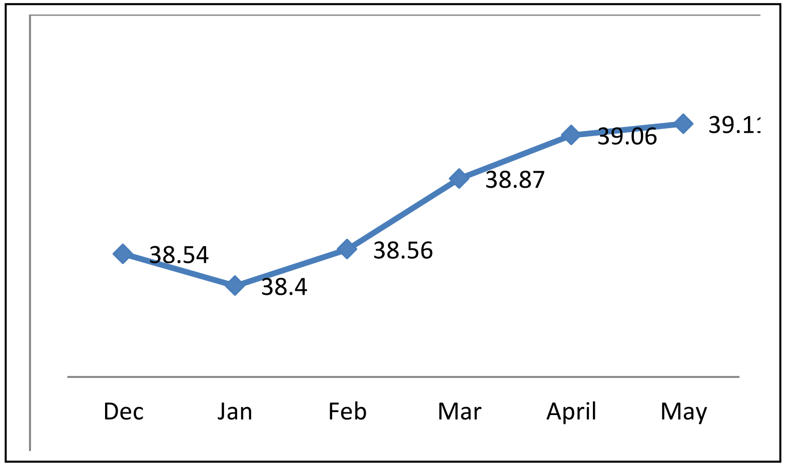

Fig. 5.25. Monthly averages of rectal temperature in eight cows

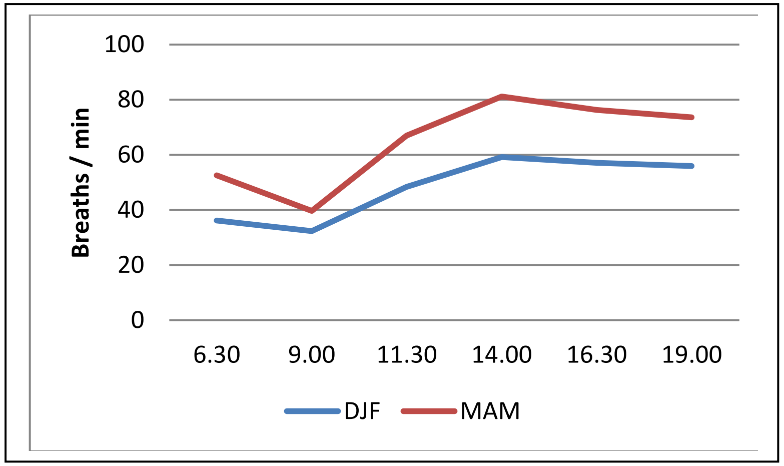

Fig. 5.26. Daily variations of mean respiration rate in two seasons

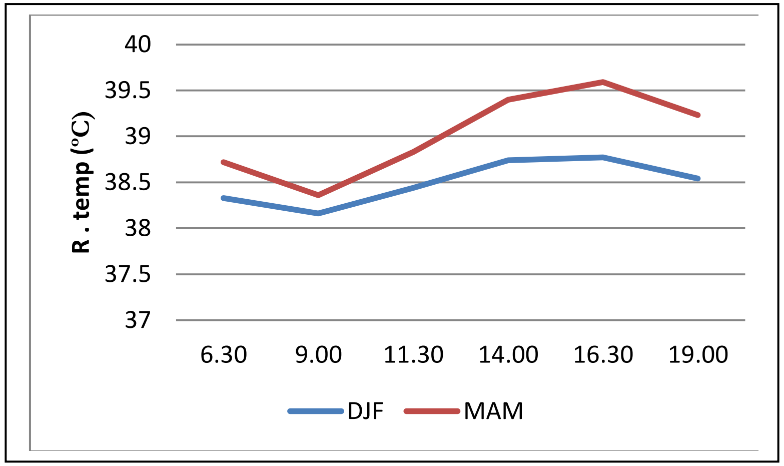

Fig. 5.27. Daily variations of mean body temperature in two seasons

the effort for reduction of active breathing, BT continued to increase until the AbT declines further so that heat dissipation by passive mechanisms becomes more effective (Rashamol *et al.,* 2018). Same mechanism can be expected in the case of monthly variation as well. Rhoads *et al.* (2009) observed that in case of TS exposed cows, increased RT was maintained for longer duration and the strain continued even up to 24 hours. Bolocan (2009) found that RT and RR increased with AbT so that RR at 4 pm was 3 to 3.5 folds than at 7 am and both parameters showed highly significant variation between the two timings.

Highly significant positive correlation of RR and RT with HSP 70, THI, AvT, AvRH, MnT and Min RH (Table 4.36) indicates direct regulation of these physiological variables by the TS.Torres-Junior *et al.* (2008), Gaughan, *et al.* (2013)and Maibam *et al.* (2017) also reported a moderate correlation of BT with HSP 70. While cortisol did not show significant correlation with RR or RT, showing its regulation predominantly by stress factors other than TS. Elevation of RT beyond normal limits causes AI failure and lowered fertility associated with TS and seasonality (De-Souza *et al.,* 2016; Kristyna *et al.,* 2017).

An increase in RT by 1°C occurring 12 h post-insemination has been found to decrease the pregnancy rates by 16 per cent (Ulberg and Burfening, 1967). Gwazdauskas *et al.* (1973) reported an increase in uterine temperature of 0.9 °F (0.5 °C) on the day of insemination and one day afterresulted in a decrease of conception rate by 13 and 7 per cent, respectively. Badinga *et al.* (1985) attributed decreased conception rates of lactating cows to their inability to maintain normal BT at high environmental temperature of around 30 °C (Allen *et al.,* 2009).

5.2.9. Ultrasonographic Findings on Ovaries

The LRST model movable trevis designed and developed as part of this study (Kutty *et al.,* 2019) was used throughout for USG of the animals and blood collection in their respective standing space causing least disturbance to the animal. More than saving time, minimal effort for restraining and ensuring safety of the scanner, examiner and the attendant, the movable trevis fabricated for the study enabled to avoid stress of handling and translocation of the animal. Since the study was to assess the influence of one form of stress, it was highly essential to avoid confounding stress factors to the possible extend and was materialized by using the movable trevisthroughout the study.

Plate 7.a. LRST model trevis with Ultrasoundequipment in position

Plate 7.b. Ultrasound scanning operation on the cow restrained using movable trevis

5.2.9.1. Follicular Growth

Irrespective of the season, large numbers of small follicles (Av. 145.66) were present on ovaries (Table 4.37a) and the number was comparatively high (163.00) during MAM and lowest during SON (114.33). However, the difference was statistically non-significant. Number of medium (Av. 43.00) and large sized (45.00) follicles were more or less equal throughout the study period and extra large follicles were very less (1.67). Mean numbers of small, medium and large follicles detected at each scanning also did not show significant variation between seasons (Table 4.37 b). There exists difference of opinion on the development of small follicles (3 to 5 mm) as increasing, decreasing and no difference in number, in response to TS (Badinga *et al.* 1993; Trout *et al.*,1998; Wolfenson *et al.*, 2000).

Torres-Junior *et al.* (2008) reported that TS did not affect follicular recruitment so that the population of >3 mm follicles and pattern of follicular growth was unaffected. This indicates that follicular recruitment and growth is taking place irrespective of the season in cattle and is regulated by various factors acting at intra ovarian level (Wolfenson *et al.*, 1995;Wilson *et al.* 1998). Within the season, numbers of different types of follicles varied significantly concurring with the report of Wolfenson *et al.* (1995).

Early phases of follicular growth are little affected by climate and are mainly controlled by factors other than climate. At the same time climate influences advanced phases of follicular growth as evidenced by the significant variation of large follicles across seasons. TS was found to increase the number of large follicles leading to follicular co-dominance as well as enhanced the growth of few larger follicles resulting increased diameter of the follicles (Wolfenson *et al.*, 1995; Torres-Junior *et al.*, 2008).

Across seasons, all types of follicles were more during MAM, even though the difference was significantly high only for large sized follicles. This indicates that climate also plays regulatory role on the pattern of large follicular growth (Kumari and Pampana, 2015). Lowest numbers of extra large follicles were in DJF, while all other follicle types were lowest during SON, even though the difference was significant only for large follicles.

MAM is the period of maximum TS and more numbers of all types of follicles during the period indicates enhancement of follicular growth by TS and is in agreement with the report of Torres-Junior *et al.* (2008).TS wasreported to cause an increase in the level of

FSH and decreased inhibin, leading to enhancement of follicular growth in cows during summer and autumn (Roth *et al*., 2000). Reduced expression of oestrus signs and resultant late detection of summer causes delayed breeding and inadequatestimuli for ovulation so that follicles continue to grow and attain larger size than other seasons.

Lopez-Gatius *et al.* (2005a) reported that ovuatory failure and persistence of pre ovualtory follicle is more during warm season. This can be attributed to the poor dominance of follicles recruited earlier as reported by De-Rensis and Scaramuzzi (2003) so that more number of smaller follicles grow simultaneously and leads to failure of ovulation, co-dominance, multiple ovulations, cyst formation and so on (Lopez-Gatius,*et al.*, 2005a; De-Rensis, *et al.*, 2015). Eventhough follicular growth is more during MAM, oestrus detected were less during the season attributable to poor manifestation and detection caused by functional incompetency of the dominant follicles.

HSP 70 showed significant correlation with counts of medium and large follicles (Table 4.38), indicatingthat TS increases the presence of such follicles, which is in agreement with the earlier finding of more follicular growth during periods of TS (Torres-Junior *et al.*,2008). Positive correlation of THI and count of medium follicles also indicates enhancement of follicular growth during summer as reported by Roth *et al*. (2000).

Level of feeding and BCS was better during MAM as discussed earlier and the positive association of these factors with follicular growth might have also contributed to better growth of the follicles during summer, since adequate nutrition and BCS are essential factors for the enhancement of follicular growth.

Serum cortisol did not show any correlation with follicular growth pattern in the present study. This finding agrees with Silanikove (2000), who reported reduced cortisol concentration in cows under chronic TS. However, there are many reports on the role of cortisol and other gluco corticoids on the ovarian follicles during luteinization.

5.2.9.2.Presence of Corpus Luteum

Detection at USG and size of functional or recent CL did not show any significant variation between seasons (Table 4.39) and also there was no association with climatic factors or other stress associated parameters. Larger size of the follicles due to longer persistence and associated functional incompetence has been reported to cause a sustained reduction in plasma P4level, contributing to lower conception rate of AI during TS (Goeseels and Kastelic, 2003).

Average size of the RCL was found to be much larger during JJA, even though the variation was not significant. This can be attributed to increased size of ovulatory follicle caused by longer persistence of previously emerged second wave follicle as reported by Wolfenson *et al.* (1995). Similarly, RCL size was found to have significant association with oestrus detected and AI done, because presence of RCL of previous cycle ensures previous ovulatory oestrus.Also there was positive association ($P<0.05$) between the presence of functional CL and number of small follicles indicating functional status of the ovaries.

5.2.9.3 Ultrasonography during Oestrus

Repeated USG at oestrus and subsequent day of oestrus mainly for comparing the follicular growth showed non-uniformity of the follicular growth pattern (Table 4.40). The ovarian activity indicated by follicular size was non-significantly higher on Day 1; whereas the total number of follicles was more on Day 0. Among the ovaries, right ovary was more active bearing largest follicle more often.

Increased size of the second largest follicle on Day 0 compared to Day 1 agreed with the earlier finding of sub-ordinate follicles growing more during the TS prone seasons reported by Wolfenson *et al.* (1995). The size of first subordinate follicle showed significant positive correlation with AvT and THI in support of the above inference and is in agreement with the report of Wolfenson *et al.* (1995) and De-Rensis *et al.* (2015). Other follicular characteristics on Day 0 and Day 1 did not show striking differences between seasons, and also there was no significant correlation with other climatic and biological factors.

5.2.9.4. Ultrasonography for Detection of Pregnancy

Number of times USGperformed for pregnancy diagnosis varied between seasons depending upon the number of AI during previous months. During MAM, only six scanning was done as against 26 in DJF. Out of total 64 scanning attempts, 47 were repeatedly done in 19 animals conceived as against 17 in non-conceived animals as shown in Table 4.42.

5.2.9.5. Embryonic Death

Embryonic mortality is more likely to occur within 35 days after conception in cattle (Das *et al.*, 2016). Even though more embryos die before 16 days, goes unnoticed because of non-alteration of cycle length. Embryo loss is more between 16 to 25 days, which in turn manifested as delayed return to service, but cannot be detected otherwise. Embryos could be easily observed using USG at 25 days and loss thereafter was easily detected by repeated examination. Out of 19 animals found to have positive signs of conception at 25 days, four of them found to have lost their embryos by 35 days.

The embryonic loss was more in SON and Nil detected during MAM, even though the numbers of pregnancies were very less to make valid conclusions. Low rate of embryonic death during MAM - the period of maximum TS can be attributed to low number of insemination and conception during the period. Even though follicular activity was more during MAM, very few oestrus were detected, and the conception rate was better since only few animals with very prominent changes were bred from a pool of many undetected. Embryonic survival also might have benefited the same way. Adaptation of crossbred animals also can be thought of contributory to better conception and lesser embryonic death during summer as mentioned by El-Tarabany and El-Tarabany (2015)

5.2.10. Serum SampleAnalysis

Frozen stored serum samples were analysed for HSP 70 at first among others, in order to avoid the chances of denaturation by repeated freeze thaw cycles. Serum cortisol was also analysed same day on the thawed samples. All the samples were subjected to ELISA in duplicate wells and thawing was done considering the number of wells needed for standards, controls, blank and so on .

5.2.10.1. Stress Associated Biological Factors

i. Serum HSP 70Level

HSP 70 levels were estimated in serum samples collected at weekly intervals during the pre-service period and also in post-service samples. HSP 70 was considered as the major determinant of TS influence on the animal (Kumar *et al.*, 2015). Since post-service samples were collected at weekly intervals in continuation of the pre-service samples, except few days of delay in starting the collection after the service, these samples were also tested for HSP 70 for better representation of the entire period of study. Thus HSP 70 levels were tested in 544 samples as shown in Table 4.43.

Mean value of HSP 70, even after excluding extreme values was higher (3.06 ± 0.07 ng/mL) than the normal levels expected for cattle without exposure to TS (0.29 ± 0.04 to

2.33 ± 0.47 ng/mL), and falls within the range (2.37 to 8.2 ng/mL) reported for cattle under TS (Archana *et al.*, 2017). Comparison of the monthly patterns of HSP 70 and cortisol are showed in Fig. 5. 28. While HSP 70 showed somewhat continuous variation, cortisol levels were highly irregular between months. Zarina (2016) reported more consistent variation of cortisol level across seasons which can be due to thatstudy on castrated male calves, unlike dairy cows in the present study, which are exposed to various stress factors.

As shown in Table 4.44., HSP 70 levels were maximum during April (7.04 ng/mL) and minimum in September (1.59 ng/mL) and the pattern of variation somewhat corresponds to that of AbT($P<0.01$), which is in agreement with the report of Mishra *et al.* (2011) and Zarina (2016). However, cortisol levels were highest in March (11.49 ng/mL) and lowest (4.81 ng/mL) in August, both being one month ahead of that of HSP. This difference in the pattern of cortisol from that of HSP 70 may be due to the involvement of other stress factors as well in regulating the cortisol levels.

Between seasons, there was highly significant variation ($P<0.001$) of the HSP 70 level with the highest during MAM, while the levels were not much different during other three seasons (Table 4.18), even though the mean values of each season varied significantly from others (Fig. 5.16). In spite of high THI to cause mild to moderate TS, almost same levels of HSP 70 during the three seasons can be attributed to the adaptation of the animal (Mishra and Palai, 2014; Archana *et al.*, 2017), whereas higher level of stress during MAM induces more secretion of HSP 70 to repair the cellular damage.

Between half years also there was highly significant variation ($P<0.001$) with higher levels of HSP 70 during long day and non-rainy half years, where in the AbT is significantly higher than the other half year counterparts. Thus HSP 70 level forms an important indicator of TS and correlation of HSP 70 with each of the study parameters and weather parameters are already discussed under each sub - section. .

ii. Serum CortisolLevel

Mean±SE cortisol level (8.27 ± 0.72 ng/mL) was within the normal range (7 to 37 ng/mL) even though value was slightly higher the expected mean value (4.5to 5.1 ng /mL). Serum cortisol varied significantly ($P<0.001$) between seasons with maximum during MAM. Wanker *et al.*, (2014) also reported significant elevation of cortisol levels with elevation of AbTfrom 25 to 35^{o}C. However, the pattern of variation was quite different from that of HSP

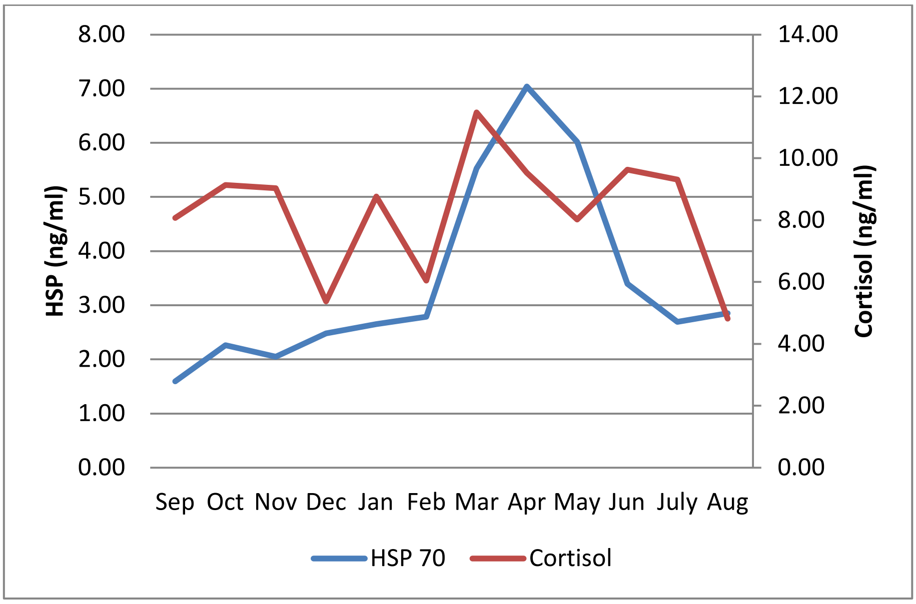

Fig. 5.28. Monthly pattern of HSP 70 and cortisol

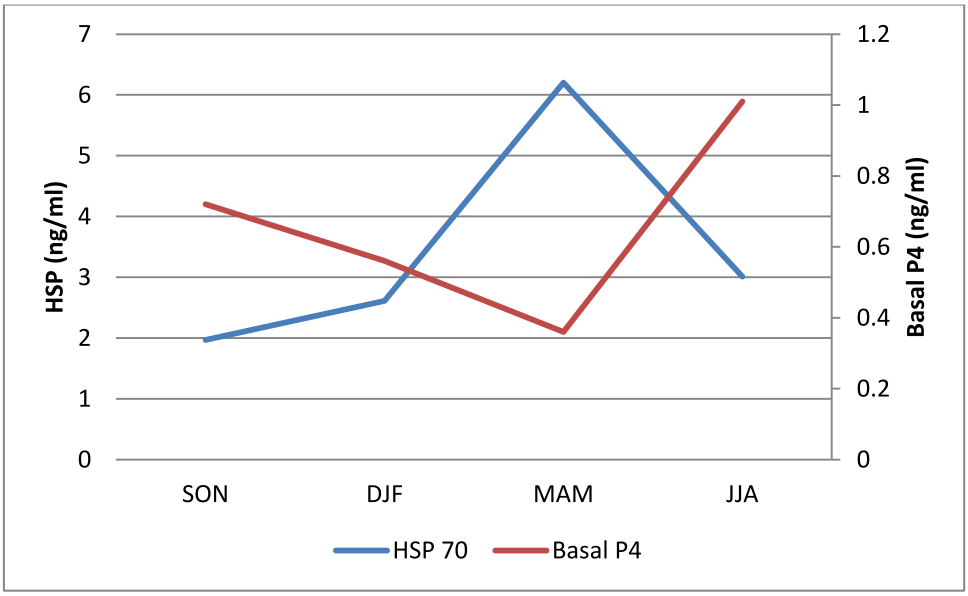

Fig. 5.29. Variation in basal Progesterone and HSP 70 levels across seasons

70 (Fig. 5. 28), showing more irregular pattern of variation between months. Between half years of long and short days, there was significant variation of cortisol level ($P<0.05$) with higher levels during long day period, whereas the variation was non-significant between rainy and non-rainy half years (Table 4.45).

Cortisol level was found to have significant correlation with weather parameters such as MxT, AvT ($P<0.01$), MxTHI and THI ($P<0.05$). De-Rensis *et al.* (2017) also found a direct effect of TS on the secretion of cortisol and prolactin during summer. However, there was no correlation of cortisol with HSP 70 in the present study indicating difference in the regulation of both these factors. Even then, influence of cortisol on various study parameters were assessed and discussed in respective sub sections.

Difference in the pattern and strength of correlation indicates that cortisol levels are not directly controlled by climate, as evidenced by no significant difference in the levels of cortisol at 30^{o}C and 40^{o}C in adult buffaloes (Wanker *et al.*, 2014) and hence does not solely represent TS. Since cortisol level varies with various stress factors, comparison of cortisol levels with other study parameters will be useful to understand the extend of simultaneous influence of general stress factors along with TS, while HSP is more specific as a biological indicator of TS.

iii. Serum MDA Level

Serum MDA levels compared between DJF and MAM (Table 4.46) showed highly significant ($P<0.001$) variation. High MDA level of summer indicates the occurrence of oxidative damage at cellular level during the period as against post monsoon (Sakatani *et al.*, 2012b). There was significant positive correlation of MDA with daily AvT, MnT and THI indicating the influence of TS in regulating MDA level as reported by Guo *et al.* (2018).

Highly significant positive correlation of MDA with physiological indicators of TS such as RR, RT and HSP 70 indicates that variation in the levels of MDA is contributed by TS (Hansen, 2009). At the same time, there was more correlation of MDA with cortisol than HSP (Table 4.47), which indicates that MDA levels are more influenced by other stress factors than TS, causing oxidative damage in tissues as reported by Kumar *et al.* (2012).Higher levels of MDA simultaneous with TS indicators can be attributed to the increase in the extent of oxidative damage at the cellular level, which is considered as one of the major mechanism of TS leading to impaired fertility (Hansen, 2009).

5.2.10.2. Reproductive Hormones

i. Oestradiol Level

Oestradiol levels on Day 0 and Day 1 of oestrus varied significantly between seasons with the highest levels during JJA and lowest in SON (Table 4.49). Since E2 levels can be correlated with the intensity and duration of oestrus signs, elevated E2levels of JJA together with almost equal levels on Day 0 and Day 1 can be attributed to prolongation of oestrus, even though external signs were lacking. However, number of larger follicles and size of the dominant follicle was comparatively low during JJA than other seasons (Table 4.41).

Oestradiol levels across seasons were related to growth pattern of follicles on the ovaries. Starting from SON, all the four categories of follicles increased toreach maximum number in MAM and E2 level also followed the same pattern. However, follicle number showed a decrease in JJA even though E2 level further increased. This can be attributed to increased secretory activity of follicles regained after the summer stress. TS causes increase in size and number of larger follicles with lowered functional competence especially during summer, concurring with the report of Wolfenson *et al.*(1995). Accordingly, lowering of AbT during JJA might have resulted increased production of E2 by the dominant follicles.

In spite of elevated oestradiol levels in JJA, number of oestrus detected was not much increased and is attributable to the lack of behavioural signs already prevalent in the herd (Nasir and Kutty, 2004). Shifting of oestrus exhibition from daytime to night hours as reported by Bolocan (2009) also might have caused lesser detection even though prolongation of E2 secretion to cause equal levels on Day 0 and Day 1.

Untimely detection of oestrus due to lack of behavioral signs can lead to the variation of E2 level between Day 0 and Day 1. Comparatively low level of E2(3.19 pg/mL) on Day 0ogether with elevated level on Day 1 (3.65 pg/mL),as in SON can be attributed to early detection of oestrus, so that E2 level continues to increase between Day 0 and Day 1. Reduction of E2 level from Day 0 to Day 1 can be due to late detection or early arrest of oestrus as seen in DJF (5.16 to 5.04 pg/ml) and MAM (5.28 to 4.96 pg/mL). Increase of BT and DM intake causes rapid metabolism of steroid hormones in the liver so that E2 level remains low and decreases rapidly in high producing animals as well as cows exposed to TS (Abdalla *et al.*, 2017).

Since none of the animals were showing prominent behavioural signs of oestrus, accuracy of heat detection was low so that actual onset of signs or even pro-oestrus could not be distinguished by human observation alone (Bolocan, 2009) even though approaches like scoring for oestrus intensity was adopted as a measure to improve heat detection rates. Thus lack of behavioural signs and difficulty to distinguish the actual onset and end of oestrus period makes the interpretation of E2 level on Day 0 and Day 1 difficult.

ii. Serum Progesterone Level

During early PP period, basal level of P4present in the serum is contributed by the RCL of gestation and extra gonadal sources. Subsequent to first PP ovulation or luteinization of the follicles without ovulation, P4 level elevates depending upon the secreting ability of CL. Thus basal level of P4 during PP period and the post-service levels vary widely depending upon so many factors (Opsomer *et al.*, 1996). Hence, both the type of P4 was considered separately.

a. Post-partumProgesterone Level

Progesterone level of PP period estimated in the serum collected at weekly intervals did not show significant variation between seasons. The level was lowest (0.36 ng/mL) duringMAM and there occurred a marked elevation(1.01 ng/mL) during JJA (Table 4.50). Also there was significant ($P<0.05$) negative correlation withMxT, indicating decrease of P4 level as AbT increases, in agreement with the report of Abdalla *et al.*(2017). However, the correlation between the number of medium sized follicles and P4 level appears to be incidental since growing follicles are not involved in regulating P4 secretion.

Progesterone being highly essential for the manifestations of oestrous cycle, conception and maintenance of pregnancy, inverse relationship with AbTcan be attributed to cause many of fertility problems associated with TS. Siregar *et al.* (2017) reported significantly higher levels of P4 in animals that maintain pregnacny (2.4 to 4.2 ng /mL) as against those suffered embryonic dealth (1.93 ng/mL). Morris *et al.* (2011) reported a very low level (0.17 ng/mL) of basal P4among cows suffering stress out of chronic lameness and hence failed to ovulate with Prostaglandin injection compared to others without stress exposure and ovulated.

Higher level of basal P4 ($P<0.05$) during rainy season and negative correlation with MxTgives an indication that TS causes impairment of normal cyclical activity in animals, in agreement with the report by Opsomer *et al.* (1996). Between seasons, variations of HSP 70 and basal P4 had an inverse relationship as shown in Fig. 5.29, even though the correlation between the two parameters was non-significant. Khanna *et al.* (1995) reported the role of HSP s as physiological mediators of luteal regression based on the inhibition of P4receptors in rats. None of the other factors including cortisol and THI showed significant correlation with P4 level of PP period.

b.Post-serviceProgesterone Level

Even though the overall mean value of post-serviceP4 levels varied significantly between seasons, there was no significant variation between the three post-service intervals and also across seasons (Table 4.51). The mean values of each intervals and the overall mean value were very low compared to the expected levels of (2.0 to 9.0 ng/mL) post-serviceP4 in cattle (Ronchi *et al.* 2001; Gomez *et al.,* 2018). This might be due to the persistence of TS throughout the year as mentioned earlier. Even though majority of the reports agree with a decrease of post-serviceP4 levels among animals under stress than others (Torres-Junior*et al.,* 2008; Macias-Cruz*et al.,* 2016), there are many reports of increase or unchanged levels as well among stressed animals and the discrepancy is attributed to the different types and levels of stress factors and metabolic state of the animals (De-Rensis*et al.,*2017).

Between seasons, mean post-serviceP4 level was highest during DJF and lowest in SON with almost similar values during DJF and MAM. Between the three post-service intervals, there was continuous increase from day 7 to day 14 attributable to the functional CL present at this stage irrespective of conception. However the level dropped by Day 21, attributable to the luteolysis in non-conceived animals. However during JJA, there was continuous increase from Day 7 to Day 21, and was contributed by more number of cows conceived during the period. Across seasons, P4 level of Day 14 was highest (1.47 ng/mL) during MAM as against higher levels of Day 7, Day 21 and the overall (1.12, 1.19 and 1.18 ng/mL, respectively)during DJF (Table 4.51). This may be due to the detection of only few oestrus with prominent signs as mentioned earlier and larger size of the FCL during MAM compared to other seasons.

During SON,P4 levels of Day 14, Day 21 and the overall had the lowest value than other seasons. This can be attributed to more non-conceived animals from MAM and JJA bred in this season, so that mean P4 level remains low in agreement with the report byGomez *et al.* (2018). Similar variation of P4 level between seasons was also reported by Wolfenson *et al.* (2000) and Ronchi *et al.* (2001).

Comparison of post-serviceP4 levels for conceived as well as total animals showed a similar pattern of variation as shown in table 4.52. There was significant variation of the mean P4 levels across seasons in conceived and total animals, whereas for non-conceived animals, the variation was non-significant between seasons. In all categories, P4 level was significantly high during DJF and MAM than other two seasons. DJF and MAM are periods with enhanced ovarian activity with more follicles and larger CL as mentioned earlier so that CL produces more P4 in conceived animals. Macias-Cruz *et al.* (2016) also reported lower levels of P4 during summer than other seasons and attributed the same to difference in the secretory function of CL.

Post-serviceP4 level did not show significant correlation with any of the climatic, physical and biological factors studied except the association with the presence of a recent RCL, this can be attributed to the adaptation out of continuous exposure to TS over the years so that almost uniform fertility is maintained across seasons.

5.2.11. Pregnancy Diagnosis

Details of USG examination for pregnancy diagnosis were described earlier. In Gr. 1 pregnancy diagnosis was carried out at 45 and 60 days by trans-rectalgynaecological palpation. In both the groups together 32 non-returns were subjected to pregnancy diagnosis on or before 45 days and further confirmation was done at 60 and 90 days by palpation. The confirmation of pregnancy after 2 months was carried out to catch any undetectable loss of early pregnancy.

5.2.11.1. Conception Rate of AI

Out of 38 AI, 19 were conceived giving a conception rate of 50 per cent, which is higher than the herd average obtained in the retrospective study. This is attributable to more attention on the time and technique of AI especially in Gr.2. animals. Even then, the conception obtained is much lower than expected standards and is attributable to features of the herd, management and the climate.Nevertheless, number of animals bred was very low especially during MAM and JJA,resulted from reduced number of oestrus and was attributableto immediate and delayed effects of TS during these periods. Heat stress has been reported to lower the conception altering the steroid profile and oocyte quality as reported by Pavani *et al.* (2015) and Abdalla *et al.* (2017).

Between seasons, conception rate was much better during JJA and low during MAM. Boni *et al.* (2014), El-Tarabany and El-Tarabany (2015) and Pavani *et al.* (2015) also rerported reduced conception during summer months when the THI increased from Low to High levels, even though crossbreds were less affected (El-Tarabany and El-Tarabany, 2015). However, total AI done in the study is inadequate to make valid conclusions on success rate of AI.

Further, pattern of conception rate across seasons observed in the prospective study deviated from that of retrospective study, wherein the highest conception rate (though non-significant) was during MAM. Such a difference is again attributed to the reduced number of AI during prospective study to make valid conculsions.

5.2.11.2. Pregnancy Loss

Out of 19 cows conceived, 4 suffered pregnancy loss during the early stage itself (within 35 days of conception). Four out of 19 formed a larger rate of pregnancy loss and was distributed in four seasons with more numbers in SON, nil in MAM and equal numbers in DJF and JJA.In the present study, EED was relatively high during SON (40.00 %) which is due to low number of total conceptions. Overall the EED rate was 21.05 per cent and is in agreement with earlier studies giving a high variability in the proportions of EED ranging from 7.2to 29 per cent (Silke *et al.,* 2002; Santos *et al.,* 2004;Abdalla *et al.,* 2017).

There are many studies reporting increased risk of early pregnancy loss in summer primarily caused by heat stress (De-Rensis*et al.,* 2015). Cows inseminated at a THI of >75 had a high risk of EED, abortion and still birth compared to the low risk for AI at THI <65 (El-Tarabany and El-Tarabany, 2015; Abdalla *et al.,* 2017). In present study, such association was not seen since the numbers are very inadequate to make valid conclusions.

The major mechanism of causing EED by TS is through P4 deficiency concurring with the report of De-Rensis *et al.* (2017). In the present study also post service P4 levels were lowest during SON and high during MAM corresponding to the pattern of EED noticed. Siregar *et al.* (2017) reported a P4 level of 1.93 ng/mL in cows that suffered early embryoic death as against 4.2 ng/mL in those normally maintained the prgnancy

5.2.12. Adaptation to Thermal Stress

Adaptation to living conditions is a fundamental biological phenomenon for existence. Since the study animal herd was continuously exposed to TS, adaptation to the prevailing conditions might have occurred over the years as explained by Wanker *et al.* (2014) based on the lack of notable difference in cortisol levels of 40^{o}C and 30^{o}C upon continuous exposure.Besides immediate responses to survive a rapid exposure to TS, long term acclimatization is also taking place which essentially involve endocrine, neuro-endocrine and behavioural responses that favour survival (Sanin *et al.,* 2016), and at the same time leads to inhibition of the reproductive process (El-Tarabany and El-Tarabany, 2015). Thus as the consequences of long term adaptation mechanisms, overall fertility of the herd has decreased resulting the lowered reproductive performance.

The effects of TS on livestock can be minimized *via* adopting suitable scientific strategies such as physical modification of the environment, nutritional management and breeding interventions favouring selection for adaptation to TS. Among cows exposed to high AbT, crossbreds were able to tolerate better than high yielding exotic breeds, even though accompanied by reduction of production potential and reproductive performance (El-Tarabany and El-Tarabany, 2015). Even though there is breed diffrences, there exists the possibility that genetic adaptation to TS in the context of worsening climate can lead to further reduction of the reproductive performance (Hansen, 2009).

Physical features, productivity levels and reproductive performance favouring survival are becoming more important in the context of upcoming climate change and adaptation to adverse climate should be given due consideration in the breeding strategies. In this context, HSP 70 forms a potential biomarker of adaptation to adverse climate especially TS (Deb *et al.*, 2015; Maibam *et al.,* 2017) as its expression has been correlated with TS resistance (Feder and Hofman, 1999, Mishra and Palai, 2014; Rajoriya *et al.,* 2014).

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SUMMARY

6.SUMMARY

Increased milk production of dairy cows has been found toenhance TS because of the simultaneous rise of metabolic heat production. Elevation of climatic stress factors attributable to climate change amplify the TS further affecting heat dissipation from the body and has been found to cause considerable reduction of the reproductive performance in these animals. In this context, present study was carried out with the objectives such as, (1) To elucidate the influence of TS on reproductive performance of crossbred dairy cows and (2) To identify suitable biological markers for timely intervention to minimize the impact of TSreproductive performance.

The study was carried out at the dairy farm of LRS located at Thiruvazhamkunnu in Palakkad district, belonging to Kerala Veterinary and Animal Sciences University.The study involved,(a) retrospective assessment of the reproductive performance of crossbred dairy cows in comparison with weather parameters over a period of six years, and (b) prospective investigation of the impact of climatic stressfactors round the year on various production and reproduction parameters of early PP cows.

Various study parameters were compared between seasons in order to assess the combined effects of climatic factors contributing TS. Classification of seasons into four quarters of three months each namely north east monsoon (SON), post monsoon (DJF), summer (MAM) and southwest monsoon (JJA) were followed.

Retrospective data of weather parameters and reproductive parameters of the herd were collected from September 2013 to August 2019 and analysed for the descriptive details, variation between monthsand seasons and the interrelationship between TS factors and reproductive performance of the animals.

Weather parameters such as AvT, MxT, MnT and THI had significant ($P<0.001$) variation between seasons with the highest attained during summer and the lowest of AvT and MxT in JJA and lowest MnT and THI during DJF. However, RH was highest during JJA and lowest in DJF influenced by the occurrence of rainfall.

Comparison of animal stock under different breeding categories did not show significant variation across seasons, in spite of variation significant ($P<0.01$) between years.

Most of the breeding management parameters such as oestrus detected, total number of AI, proportion of oestrus animals bred and monthly calvings varied between seasons with the lowest performance during summer, but the variations were statistically non-significant. This can be attributed to the variations of management strategies across the years. The conception rate of AI was slightly better during summer, contrary to the earlier reports, attributable to the reduced number of oestrus detected so that only few animals with prominent signs could be inseminated leading to better conception.

Fertility indices studied such as age at first calving, days to onset of PP oestrus, service period, inter-oestrus interval, AI per conception, calving to conception interval and inter-calving interval showed non-significant variation between seasons over the six years. Even though more animals were inseminated during JJA, conception achieved was low and attributed to the delayed effect of TS affected during summer.

DAI performed as a management measure for prolonged oestrus formed 26.0 per cent of total oestrus with breeding done and conception rate of double AI was better (42.6 %) than single AI (34.0 %) over the years. Across seasons, conception rate of DAI was lowest during JJA, attributable to delayed effect of TS, and significantly better during summer contributed by selective breeding as mentioned earlier.

Between seasons, even though there was highly significant variation of AbT, RH and THI, breeding related parameters did not show significant variation. Also there was no significant correlation between these parameters and is attributed to the inconsistency of monthly pattern between years so that the variability between seasons gets nullified.

Between the two periods of low (72 to 78) and high THI (More than 78) as well, there was no significant variation for breeding related parameters. Together with lowered overall figures, maintenance of more or less uniform reproductive performance even at high THI is believed to be due to adaptation of the crossbred animals, acquired through continuous exposure to adverse climate of mild to moderate TS throughout the year.

Even though weather parameters did not vary significantly between years, (yearly average of AbT increased by 0.6 degree in 6 years), breeding related parameters showed significant variation between years and significant association with weather parameters such as MnT, AvT, MxT, MnRH, AvRH, MxRH and THI across the years. This can be attributed to possible variations of management strategies of the farm between years.

Above findings imply that in spite of THI values exceeding the limit for thermal comfort of dairy cattle throughout the year, significant variation of the breeding related study parameters across the years and significant variation of weather parameters between seasons, breeding related parameters were more or less uniform across seasons. However, the overall reproductive performance was very low based on expected standards indicating harmful effects of TS throughout the year.

Adaptability of theanimals to the prevailing adverse climate through continuous rearing at the same place and consequent passive selection over the years might be the reason for reduced overall reproductive performance, maintaining almost uniform level across the seasons and lack of a major decline during summer.

Prospective study was carried out from Sep 2018 to August 2019. PP cows were selected each month and collection of general parametersand detailed investigationswere carried out at frequent intervals. Weather parameters during the period were recorded both outside and inside the animal houses and AbT, RH and THI within the animal houses were compared with various study parameters.

Variations of AbT, RH and THI were significant between seasons with the highest in summer following the same pattern of variation as in retrospective study. Between half years of short and long days as well as rainy and non-rainy months, AbT and THI did not show significant variation even though RH was significantly high during rainy season.

In addition to the climatic factors causing TS, the response of the animals to TS was assessed based on the levels of HSP 70 and cortisol in the serum. While cortisol indicated stress out of various reasons, HSP 70 was considered more specific for TS. Mean values of these biological factors were compared between months, seasons and half years and also compared with weather parameters and various study parameters to assess the variations and influence of TS on the study parameters.

Overall mean values of HSP 70 and cortisol were higher than expected normal values. HSP 70 and cortisol values varied significantly between seasons and HSP 70 had the same pattern of variation as that of AbT and THI. At the same time cortisol variation across seasons did not show significant correlation with any of the weather parameters.

Dry matter intake was significantly high during the summer and lowest in SON compared to other seasons. HSP 70 levelwas found to have significant positive correlation with feed intake and appears to be due to increased milk yield during summer. Mild or moderate level of TS suffered by the animals was also believed to increase the maintenance requirement.

Milk yield was highest during first two months of lactation ($P<0.01$), and across seasons achieved the maximum during MAM, especially when these two periods coincide. Reduction in milk yield was associated with reduced feed intake and BCS.The involvement of stress adaptation causing no reduction of milk yield even in summer can also be thought of contributing to the phenomenon.

The BCS was significantly low during SON than other three seasons comparable with the low DM intake during the season. BCS adjusted for stage of lactation showed neither significant variation between seasons nor significant correlation with weather parameters. However, BCS was found to have significant correlation with cortisol alone indicating involvement of stress reasons other than thermal factors.

Number of oestrus detected was significantly more during DJF, contributed by favorable weather, and lowest in JJA attributable todelayed effect of TS. The numbers of oestrus detected and animals involved was not found to have any association with weather parameters and other study parameters.

Comparison of the oestrus intensity assessed based on the scoring approach did not show significant variation between seasons. The scores were better during DJF contributed by higher scores of uterine changes and mucous flow and attributable to congenial climate. Across seasons, oestrus intensity scores had more inverse relationship with cortisol, and not with HSP 70, indicating involvement of various management and social factors other than TS influencing expression of oestrus behaviour.

AI done was comparable with oestrus detection rate being more number in DJF and lowest in MAM. However, monthly average of AI done was inadequate to make valid conclusions since the study was during early PP period (upto 91 days PP). Low reproductive performance of the herd in the prevailing climate, in spite of better management and moderate productivity appears causative.

Respiration rate and RT of the animals showed highly significant (P<0.01) variation between the two seasons, months and even between each times of recording. Both RR and RT were having highly significant positive correlation with HSP 70, THI, AvT, AvRH, MnT and MnRH and indicate direct regulation of these physiological variables by the TS. While cortisol did not show significant correlation with RR or RT, may be due to regulation of cortisol by stress factors other than TS.

Routine, frequent and convenient USG examination utilizing minimum resources and causing little stress to the animal was carried out using LRST model movable trevis designed and developed as part of the study. USGof reproductive organs was performed at weekly intervals on each of the animals included under Gr. 2. In addition,USG for pregnancy diagnosis and oestrus confirmation was also done as and when needed.

Irrespective of the season, large numbers of small follicles were present on ovaries, while medium and large sized follicles were also present but lessthroughout the study period indicating the occurrence of follicular recruitment and growth during all the seasons. Across seasons, all types of follicles were more during MAM, indicating better follicular growth during summer, even though the difference was significantly high only for large sized follicles. TS cause poor dominance offollicles recruited earlier, so that more number of medium and smaller follicles are recruited to grow. HSP 70 had significant correlation with counts of large and medium follicles, indicating increase in their count with TS.

Even through follicular growth is more during MAM, oestrus detected were less during the season attributable to poor manifestation of oestrus caused by functional incompetency of the dominant follicles. Detection and size of functional or recent RCL during USG did not show any significant variation between seasons and also there was no association with climatic factors or other stress associated parameters. Average size of the RCL was found to be comparatively larger during JJA attributable to larger size of the follicle ovulated caused by its longer persistence during MAM and JJA.

Follicles observed on Day 0 and Day 1 of oestrus showed non-uniformity of their growth pattern. The ovarian activity indicated by follicular size was slightly higher on Day 1, where as the total number of follicles was more on Day 0. Variations in the size of the dominant follicle on Day 0 and Day 1 were attributed to the alterations in the time of oestrus detection rather than direct effect of TS.

Number of times early detection of pregnancy carried out by USGvaried between seasons depending upon the number of inseminations done during each of the previous months, but the variation was statistically non-significant.

Monthly pattern of HSP 70 showed consistentvariation; where as cortisol levels were highly irregular between months. The variations of HSP 70 were resembling to that of AbT with maximum during April and minimum during September. Even though serum cortisol varied significantly between seasons, there was no correlation with HSP 70. Difference in the pattern of variation and strength of correlation with weather parameters indicate that cortisol levels are not directly indicative of TS. Hence, comparison of cortisol levels with other study parameters is considered useful to assess the influence of general stress factors along with TS, while HSP 70 is more specific as a biological indicator of TS.

MDA levels of the serum compared between DJF and MAM showed highly significant variation with higher levels during summer indicating occurrence of oxidative damage at cellular level. There was significant positive correlation of MDA with AbT and THI indicating TS as the reason for the occurrence of oxidative damage. MDA also showed highly significant positive correlation with physiological indicators of TS such as RR, RT and HSP 70, indicating influence of TS on MDA levels. At the same time, there was more correlation of MDA with cortisol than HSP 70, indicating more influence of other stress factors compared to TS, causing oxidative damage in tissues.

Oestradiol levels showed different patterns of variation between Day 0 and Day 1, such as increasing, decreasing or remaining unchanged across seasons. Even though unaccompanied by adequate behavioural signs, difference in the levels of estrogen between Day 0 and Day 1 are attributed to have caused by prolongation, early and late detection of oestrus and was influenced by seasonal variations of climatic and management factors. Elevated levels of E2 are also found to have associated with growth pattern and functional competency of follicles across seasons as mentioned earlier

Progesterone level of PP period did not show significant variation between seasons. However, significant negative correlation with MxT indicated that as the AbT increases, P4 level decreases. Between seasons, HSP 70 had opposite pattern of variation as that of postpartum P4, even though the correlation was non-significant. Significantly high level of progesterone during rainy seasons and positive correlation with RH gives an indication that it is not TS alone that regulates normal cyclical activity in these animals.

Overall mean values of post-serviceP4 varied significantly between seasons. However, mean P4 levels of three post-service intervals did not show significant variation between each other and between the seasons. P4 concentration was found to be reduced by TS indicated by the low levels of P4 during MAM. After categorizing based on conception, P4 levels showed significant seasonal variation between conceived and total animals with higher values during DJF and MAM. Post-serviceP4 level did not show significant correlation with any of the climatic, physical and biological parameters studied

Overall conception rate of animals under the study was 50 per cent, which was higher than herd average obtained in the retrospective study. This was attributable to better attention at oestrus confirmation and the technique of insemination. Between seasons, conception rate was much better during JJA and low during MAM deviating from that of retrospective study. But total number of AI performed in the study is very less attributable to reduced number of oestrus and breeding for which TS can be a major contributor.

Out of 19 conceptions, four animals were found to have lost their embryos between 25 and 35 days of AI, distributed as two in SON, one each in DJF and JJA and none in MAM. Even though 4 out of 19 makes high rate of pregnancy loss (21.05 %), the total number is inadequate to make valid conclusions. Smaller numbers of insemination and conceptions might have contributed less number of embryonic death during MAM, even though it is the period of maximum TS. In addition, follicular activity and conception rate of AI was better during MAM, and reasons for the same also might have benefitted for better embryonic survival as well.

From the findings so far, none of the productive and reproductive performance oriented parameters showed prominent variation and consistent association with climatic stress factors. Since the study animals are continuously exposed to TS ranging from mild to moderate levels, gradual adaptation to the prevailing conditions cannot be ruled out. May be the consequence, overall reproductive performance of the animals are very low than expected. At the same, the low profile reproductive performance is maintained irrespective of the seasons, tolerating the adversities of climate occurring throughout the year.

Besides immediate responses for surviving the adverse effects of TS, long term acclimatization is also taking place to ensure survival and welfare of the animal within the prevailing environment, which essentially involve reduction of the reproductive rate. Thus, as the consequence of long term adaptation mechanisms, overall fertility of the herd has decreased as evidenced in this study. Also further lowering can be forecasted in context of more worsening climate and management situation.

From the findings of the study, it could be concluded that

1. The weather parameters causative of TS was prevailing throughout the year with significant variation between months, seasons and half years.
2. The animals were showing physiological response of TS indicated by significant elevation of biological indicators of stress such as HSP 70, cortisol and MDA, that maintains significant positive correlation with the major determinants of TS such as AbT, RH and THI.
3. The reproductive performance and productivity parameters did not evidence marked variations comparable with that of climatic stress factors.
4. The overall reduced performance is maintained throughout the year irrespective of the seasons,which indicate that theanimals are getting adapted to moderate adversities of the environment, due to chronic exposure.
5. More focused investigations are needed to understand the characteristics, trends and productivity prospects of these adaptations especially in the context of upcoming adversities of the climate change.

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6.a. FUTURE PERSPECTIVES

More focussed investigations are needed to

1. To develop rapid detection tests based on the levels of HSP 70 to be used as early indicator of TS in farm animals
2. Identify suitable interventions to minimize the fertility impact of TS together with maintenance of productivity
3. Develop preventive management strategies depending upon the type of animal so that reproductive performance remain unaffected
4. Formulate suitable means to forecast the climate change related alterations in the environment both in short term and long term
5. Identify the expression features HSP and HSF genes to be incorporated as criteria for breeding to enhance tolerance to TS
6. Study the characteristics, mechanisms, trends and productivity prospects of adaptation to TS in long term
7. Promote climate resilient animal production, especially in the context of upcoming adversities of the climate change

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APPENDICES

Appendix 1.

Yearly Variation of Reproductive Parameters Over Six Years

Table 6.1.Mean ± SE of stock, breeding and fertility parameters having significant variation between years of retrospective study

Year	Years							F value
	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	Overall	
Total cows	110.42 ± 2.95^{d}	97.58 ± 0.80^{c}	92.92 ± 3.57^{bc}	79.75 ± 5.45^{a}	83.00 ± 4.24^{ab}	82.17 ± 3.67^{ab}	90.97 ± 2.61**	9.98
Cows in milk	58.75 ± 0.44^{b}	58.25 ± 0.08^{b}	59.17 ± 1.02^{b}	46.92 ± 2.39^{a}	50.17 ± 1.91^{a}	59.25 ± 4.27^{b}	55.42 ± 1.30**	6.14
Dry cows	51.67 ± 2.94^{c}	39.33 ±0.73^{b}	33.75 ± 4.25^{b}	32.83 ± 3.22^{b}	32.83 ± 2.64^{b}	22.92 ± 2.57^{d}	35.56 ± 2.09**	10.59
Breedable dry cows	17.22 ± 0.98^{c}	13.11 ± 0.24^{b}	11.25 ± 1.42^{ab}	10.94 ± 1.07^{ab}	10.94 ± 0.88^{ab}	8.67 ± 1.27^{a}	12.02 ± 0.67**	7.74
Breedable cows in milk	19.58 ± 0.15^{b}	19.42 ± 0.03^{b}	19.72 ± 0.34^{b}	15.64 ± 0.80^{a}	16.72 ± 0.64^{a}	19.75 ±1.42^{b}	18.47 ± 0.43**	6.14
Breedable heifers	34.58 ± 1.85^{b}	24.33 ± 1.19^{a}	51.08 ± 4.07^{d}	41.50 ± 4.55^{bc}	38.08 ± 0.34^{bc}	43.50 ± 0.74^{cd}	38.85 ± 1.97**	11.44
Total breedable females	71.39 ± 2.20^{bc}	56.86 ± 1.17^{a}	82.06 ± 5.14^{c}	68.08 ± 6.36^{ab}	65.75 ± 1.33^{ab}	71.92 ± 2.21^{bc}	69.34 ± 2.05**	5.15
Total adult females	145.00 ± 3.67^{b}	121.92 ± 1.31^{a}	144.00 ± 7.39^{b}	121.25 ± 9.99^{a}	121.08 ± 4.13^{a}	125.67 ± 3.97^{a}	129.82 ± 3.03**	3.92
Pregnant cows	31.79 ± 2.81^{abc}	24.46 ± 0.45^{a}	26.44 ± 1.07^{ab}	33.11 ± 2.66^{bc}	37.56 ± 4.18^{c}	39.26 ± 3.03^{c}	32.10 ± 1.48**	4.81
Pregnancy. per adult females	21.90 ± 1.77^{ab}	20.10 ± 0.29^{a}	18.65 ± 1.54^{a}	27.51 ± 1.55^{bc}	30.99 ± 3.04^{c}	31.29 ± 2.39^{c}	25.07 ± 1.28**	8.15
Breeding Proportion (%)	92.50 ± 3.04^{d}	89.47 ± 2.68^{cd}	77.42 ± 4.05^{bc}	70.98 ± 3.40^{ab}	58.37 ± 2.49^{a}	62.54 ± 7.44^{a}	75.21 ± 3.05**	10.97
Inter-oestus interval (days)	44.40 ± 3.36^{b}	44.86 ± 2.41^{b}	37.86 ± 2.90^{ab}	37.27 ± 2.54^{ab}	35.49 ± 0.81^{a}	36.47 ± 1.37^{a}	39.39 ± 1.17*	**2.97**
Total conception	3.83 ± 0.52^{a}	5.08 ± 0.16^{ab}	8.67 ± 1.55^{c}	6.58 ± 0.82^{abc}	6.00 ± 0.72^{abc}	7.25 ± 1.15^{bc}	6.24 ± 0.47*	**3.27**
Double AI done	2.50 ± 0.82^{a}	3.33 ± 0.68^{a}	4.50 ± 1.17^{abc}	5.83 ± 0.52^{bc}	6.08 ± 0.72^{c}	3.50 ± 0.40^{ab}	4.29 ± 0.39*	**3.59**
Double AI conception	1.25 ± 0.48^{a}	1.67 ± 0.36^{ab}	2.33 ± 0.58^{abc}	2.92 ± 0.37^{bc}	3.00 ± 0.27^{c}	1.83 ± 0.29^{ab}	2.17 ± 0.20*	**3.02**
Single AI conception	2.58 ± 0.44^{a}	3.42 ± 0.50^{a}	6.33 ± 1.28^{b}	3.67 ± 0.69^{ab}	3.00 ± 0.61^{a}	5.42 ± 1.29^{b}	4.07 ± 0.42*	**2.85**
Double AI proportion	16.27 ± 6.38^{a}	17.04 ± 4.20^{a}	19.44 ± 2.54^{a}	38.33 ± 4.06^{b}	39.06 ± 5.07^{b}	25.81 ± 4.94^{ab}	25.99 ± 2.60**	4.94
AI per conception	2.36 ± 0.25^{a}	3.69 ± 0.25^{b}	3.20 ± 0.21^{b}	2.43 ± 0.29^{a}	2.26 ± 0.18^{a}	2.01 ± 0.21^{a}	2.66 ±0.15**	7.48
Conception rate	25.36 ± 6.68^{a}	24.50 ± 3.13^{a}	32.63 ± 6.93^{a}	41.11 ± 2.81^{ab}	39.86 ± 3.90^{b}	56.14 ± 5.87^{c}	36.60 ± 0.15**	5.26

*. Significant (P<0.05), * *. Significant (P<0.01), Means with different superscript varies significantly between years

Appendix -2

Validation of Oestrus Intensity Scoring

Objective: To validate the proposed method of oestrus intensity scoring in cattle

Methodology: Post-partum cows confined to barns and found to have lack of behavioral signswere frequently observed for minor secondary chnges and behavioral alterations attributable to oestrus. All the detected animals were verified for breeding history, observed for associated external signs and clinico gynaecologicalfindings. Those having adequate uterine tone and cervical relaxation were inseminated.The details were recorded and all the animals irrespective of AI done,were observed on subsequent days for further signs of oestrus, until further AI or verification of the conceptionof AI later.

Table 6.2.Oestrus signs and scores allotted to each items for assessing oestrus intensity

S. No	Oestrus signs	Item score	Maximum score
1	**Behavioural changes**	20	20
2	**Breeding history**	15	15
3	**Mucous flow**	15	15
4	**Changes on External genitals**		10
	a. Vulval oedema	5	
	b. Vestibular hyperaemia	5	
5	**Cervical & uterine changes**		25
	a. Cervical relaxation	10	
	b. Uterine tonicity	15	
6	**Ovarian structures**		15
	a. Medium /large Follicle	15	
	b. Corpus luteum (Regressing)	10	
7	**Total score**		100

A score sheet was developed with due consideration of all the minor changes as shown in the Table 6.2 Adequate scores were assigned for history, secondary behavioural signs, minor external manifestations and findings of clinico-gynecological examination. The scores were finalized through repeated modification of the weightage considering the association of each of the chngers with true oestrtus verified based on the occurrence of subsequent oestrus, inter oestrus interval and conception of AI. Detailed score sheet is given in Appendix 3. A total score of 40 or more out of 100 was considered as the indication of proper oestrus and formed the criteria for performing AI.

The usefulness of the sheet was tested on 103 oestrus periods reported among 64 animals. Adequate scores were assigned as shown in Appendix .3. Any animal found to have abnormalities of reproductive function and inadequate scores were exempted from breeding and those having scores exceeding 40 were inseminated and followed up.

The score sheet developed was found to be highly useful to assess oestrus intensity and to rule out improper oestrus.

Appendix 3.

Model Score Sheet

Table 6.3. Model score sheet used for oestrus intensity assessment

1 **Behavioural signs**

					Score to be allotted		
				Score	Range	Limit	Example
Maximum 20 points	Specific signs	1	Trying to mount (animals / objects)	4	4		
		2	Standing for nudging / mounting	4	4		
		3	Characteristic vocalisation	4	4		
	Non-specific signs	1	Standing up / not lying down	2	2		
		2	Sniffing nearby cows	2	2		2
		3	Arching of back	2	2		
		4	Tail deviation	2	2		1
		5	Excitement / anxious look	2	2		
		6	Intermittent urination	2	2		
		7	Disturbing nearby cows	2	2		
		8	Reduced milk yield	2	2		2
			Reduced interest in feed	2	2		
			Total		0 to 30	20	5

2 **Reproductive history**

Maximum 15 points	Heifer		Breedable age, Not yet bred	5	5		
	Days from calving		Less than 30 days	-10	0		
			More than 30 days	5	5		5
	Previous oestrus		Oestrus 18-22 days back	15	15		
			Oestrus 38-44 days back	10			10
			Oestrus 56-66 days back	5			
			Total		-10 to 25	15	15

3 **Mucous flow from the vagina**

Maximum 15 points		1	Thick and voluminous flow seen	15	15		
		2	Thin and scanty flow	10			
		3	Seen smeared on the body / floor	7			5
		4	Small volume persisting at vulva	3			
			Total		0 to 15	15	5

4 **Changes on external genitals**

Maximum 10 points	1. Vulval oedema	1	Mild degree	2		5	2
		2	Moderate degree	3			
		3	Higher degree	5	5		
	2. Vestibular hyperaemia	1	Mild degree	2		5	
		2	Moderate degree	3			3
		3	Higher degree	5	5		
			Total		0 to 10	10	5

5 **Internal changes**

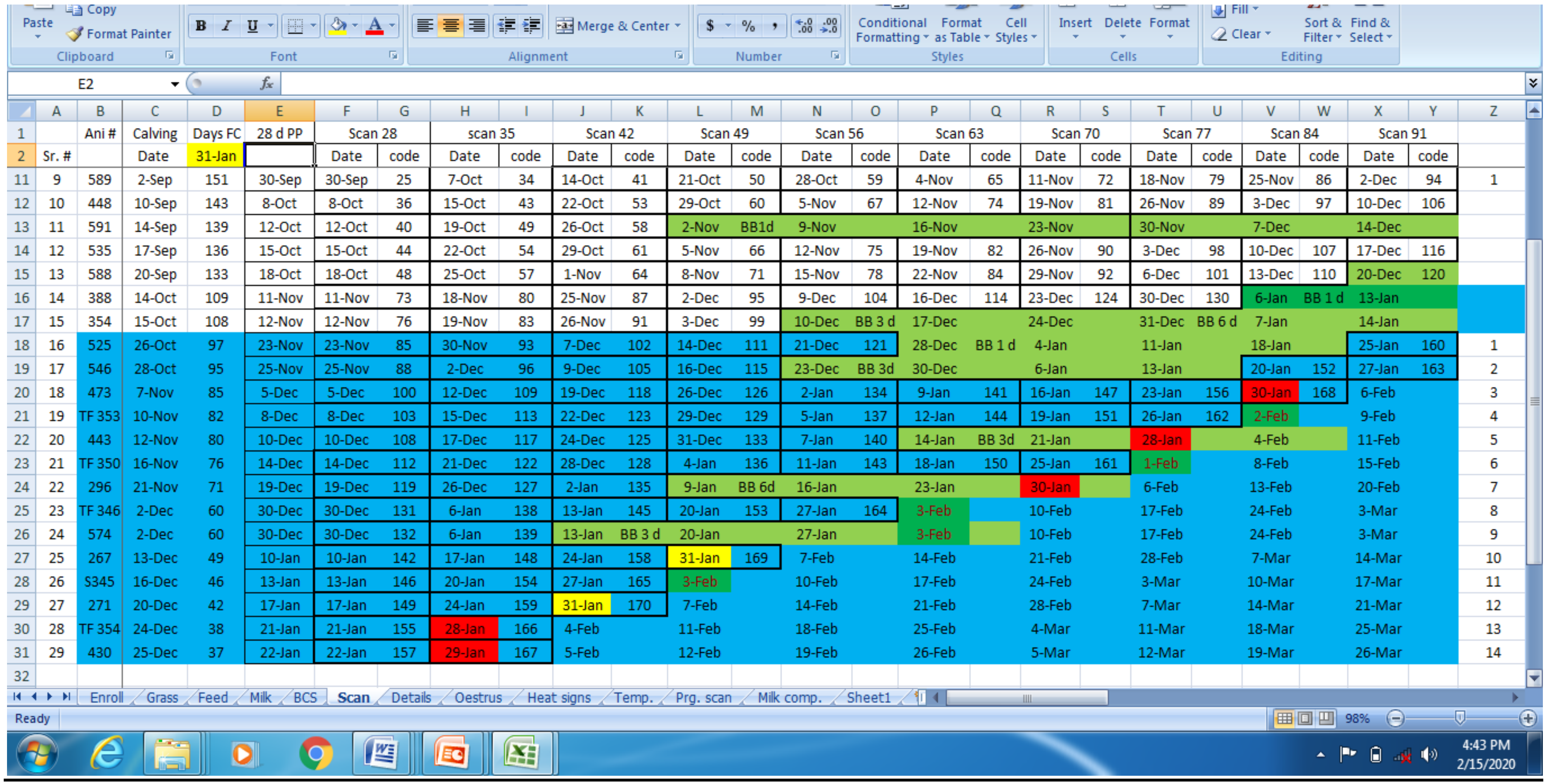
Maximum 25 points	1. Cervical relexation	1	Mild degree	5		10	5
		2	Mild to moderate	8			
		3	Moderate to high	10	10		
	2. Uterine tonicity	1	Mild degree	5		15	
		2	Moderate	10			10
		3	Higher degree	15	15		
		4	Extremely higher degree	-10			
			Total		-10 to 25	25	15

6 **Ovarian structures**

Maximum 15 points	1. Follicle	1	Larger follicle palpable	10	10		
		2	Small follicles palpable	5			5
	2. Corpus luteum	1	Recent RCL on any one ovary	10	10		10
		2	Functional CL palpable	-15			
			Total		-15 to 20	15	15
			G. Total		130	100	60

Appendix 4.

Excel Sheet Used for Work Planning



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1		Ani #	Calving	Days FC	28 d PP	Scan 28		scan 35		Scan 42		Scan 49		Scan 56		Scan 63		Scan 70		Scan 77		Scan 84		Scan 91		
2	Sr. #		Date	31-Jan		Date	code	Date	code	Date	code	Date	code	Date	code	Date	code	Date	code	Date	code	Date	code	Date	code	
11	9	589	2-Sep	151	30-Sep	30-Sep	25	7-Oct	34	14-Oct	41	21-Oct	50	28-Oct	59	4-Nov	65	11-Nov	72	18-Nov	79	25-Nov	86	2-Dec	94	1
12	10	448	10-Sep	143	8-Oct	8-Oct	36	15-Oct	43	22-Oct	53	29-Oct	60	5-Nov	67	12-Nov	74	19-Nov	81	26-Nov	89	3-Dec	97	10-Dec	106	
13	11	591	14-Sep	139	12-Oct	12-Oct	40	19-Oct	49	26-Oct	58	2-Nov	BB1d	9-Nov		16-Nov		23-Nov		30-Nov		7-Dec		14-Dec		
14	12	535	17-Sep	136	15-Oct	15-Oct	44	22-Oct	54	29-Oct	61	5-Nov	66	12-Nov	75	19-Nov	82	26-Nov	90	3-Dec	98	10-Dec	107	17-Dec	116	
15	13	588	20-Sep	133	18-Oct	18-Oct	48	25-Oct	57	1-Nov	64	8-Nov	71	15-Nov	78	22-Nov	84	29-Nov	92	6-Dec	101	13-Dec	110	20-Dec	120	
16	14	388	14-Oct	109	11-Nov	11-Nov	73	18-Nov	80	25-Nov	87	2-Dec	95	9-Dec	104	16-Dec	114	23-Dec	124	30-Dec	130	6-Jan	BB 1 d	13-Jan		
17	15	354	15-Oct	108	12-Nov	12-Nov	76	19-Nov	83	26-Nov	91	3-Dec	99	10-Dec	BB 3 d	17-Dec		24-Dec		31-Dec	BB 6 d	7-Jan		14-Jan		
18	16	525	26-Oct	97	23-Nov	23-Nov	85	30-Nov	93	7-Dec	102	14-Dec	111	21-Dec	121	28-Dec	BB 1 d	4-Jan		11-Jan		18-Jan		25-Jan	160	1
19	17	546	28-Oct	95	25-Nov	25-Nov	88	2-Dec	96	9-Dec	105	16-Dec	115	23-Dec	BB 3d	30-Dec		6-Jan		13-Jan		20-Jan	152	27-Jan	163	2
20	18	473	7-Nov	85	5-Dec	5-Dec	100	12-Dec	109	19-Dec	118	26-Dec	126	2-Jan	134	9-Jan	141	16-Jan	147	23-Jan	156	30-Jan	168	6-Feb		3
21	19	TF 353	10-Nov	82	8-Dec	8-Dec	103	15-Dec	113	22-Dec	123	29-Dec	129	5-Jan	137	12-Jan	144	19-Jan	151	26-Jan	162	2-Feb		9-Feb		4
22	20	443	12-Nov	80	10-Dec	10-Dec	108	17-Dec	117	24-Dec	125	31-Dec	133	7-Jan	140	14-Jan	BB 3d	21-Jan		28-Jan		4-Feb		11-Feb		5
23	21	TF 350	16-Nov	76	14-Dec	14-Dec	112	21-Dec	122	28-Dec	128	4-Jan	136	11-Jan	143	18-Jan	150	25-Jan	161	1-Feb		8-Feb		15-Feb		6
24	22	296	21-Nov	71	19-Dec	19-Dec	119	26-Dec	127	2-Jan	135	9-Jan	BB 6d	16-Jan		23-Jan		30-Jan		6-Feb		13-Feb		20-Feb		7
25	23	TF 346	2-Dec	60	30-Dec	30-Dec	131	6-Jan	138	13-Jan	145	20-Jan	153	27-Jan	164	3-Feb		10-Feb		17-Feb		24-Feb		3-Mar		8
26	24	574	2-Dec	60	30-Dec	30-Dec	132	6-Jan	139	13-Jan	BB 3 d	20-Jan		27-Jan		3-Feb		10-Feb		17-Feb		24-Feb		3-Mar		9
27	25	267	13-Dec	49	10-Jan	10-Jan	142	17-Jan	148	24-Jan	158	31-Jan	169	7-Feb		14-Feb		21-Feb		28-Feb		7-Mar		14-Mar		10
28	26	S345	16-Dec	46	13-Jan	13-Jan	146	20-Jan	154	27-Jan	165	3-Feb		10-Feb		17-Feb		24-Feb		3-Mar		10-Mar		17-Mar		11
29	27	271	20-Dec	42	17-Jan	17-Jan	149	24-Jan	159	31-Jan	170	7-Feb		14-Feb		21-Feb		28-Feb		7-Mar		14-Mar		21-Mar		12
30	28	TF 354	24-Dec	38	21-Jan	21-Jan	155	28-Jan	166	4-Feb		11-Feb		18-Feb		25-Feb		4-Mar		11-Mar		18-Mar		25-Mar		13
31	29	430	25-Dec	37	22-Jan	22-Jan	157	29-Jan	167	5-Feb		12-Feb		19-Feb		26-Feb		5-Mar		12-Mar		19-Mar		26-Mar		14
32																										

Appendix.5

ELISA for HSP 70 in Serum

The HSP 70 ELISA Kit works on the basis of sandwich model enzyme linked assay. The Kit included

- Micro plate - 96 well plate coated with purified anti HSP antibody
- HRP conjugated anti HSP 70 antibody for detection of HSP antibody
- Standards and standard diluent buffer
- TMB (Tetra methyl benzidine) substrate A & B to visualize enzymatic reaction
- Sample diluents, wash buffer and stop solution.

TMB reaction was catalysed by HRP to produce a blue colour that changed into yellow colour upon addition of stop solution. The intensity of yellow colour is proportional to the amount of HSP 70 captured by the plate from the sample. The optical density was read at 450 nm using microplate reader (iMark microplate reader - Bio-RAD laboratories) and the concentration of HSP 70 was obtained from the ELISA reader. The sequence of assay procedure was as follows

1. The serum samples were thawed and mixed well.
2. All diluents and buffers were brought to the room temperature before use (24°C).
3. Identified 2 wells for the controls and 10 wells for the standards (in duplicates)
4. Added 50 µl of the standard into the well for standard 1 and serially diluted using standard diluent as per kit instruction to make other standards
5. Control wells were added with 100 µl standard diluent alone
6. Sample wells were added with 40 µl sample diluent and 10 µl serum
7. Mixed gently, sealed the plate and incubated in dark for 30 min at 37 ºC
8. Wash off the well components using wash buffer in ELISA washer
9. Added 50 µl HRP conjugate into the wells except for control
10. Sealed the plate and incubated in dark humid chamber for 30 min at 37 ºC
11. Unbound conjugate was washed off with buffer in an ELISA washer (Immuno wash - model 1575, Bio Rad laboratories, CA)
12. Added 50 µl each of TMB substrates A & B into the wells except control

13. Mixed gently and incubated for 15 min at 37 ºC for the colour reaction to occur
14. Added 50 µl of stop solution and the blue colour was changed to yellow
15. Read the optical density absorbance (OD) at 450 nm in a micro plate reader (iMark microplate reader - Bio-RAD laboratories)
16. The OD values of the standards were used for plotting standard curve and HSP70 concentration for the serum samples were obtained from Microplate manager software (Bio Rad Microsoft manager 6.0, Bio Rad Laboratories, CA).

Appendix 6.

ELISA for Serum Cortisol

The ELISA Kit (Neogen laboratories) works on the basis of competition between the enzyme conjugate and cortisol in the sample for a limited number of binding sites on the antibody coated plate. The kit procedure necessitates extraction of cortisol from the serum samples before performing the assay. Kit components include

- Micro plate - cortisol antibody coated 96 well plate,
- Standards,
- EIA buffer,
- Cortisol enzyme conjugate
- Substrate,
- Extraction buffer

Ether extraction of cortisol

Ether extraction of cortisol was carried out as follows

a. Serum sample (100 µl) was taken in a glass tube and added 1ml ethyl ether
b. Closed the tube using stopper and shaken the tube for 30 seconds
c. Aspirated the liquid into 1 ml micro pipette tip and allowed the phases to separate out
d. Remove the inorganic phase carefully from the bottom with extra care
e. Transferred the organic phase alone into another tube and allowed to evaporate
f. The residue was dissolved in 100 µl of diluted extraction buffer
g. Added 10 µl of the dissolved extract into 990 µl of diluted extraction buffer
h. Assay value using the diluted extract was multiplied by 100 to adjust dilution.

Cortisol estimation

1. Allocated the wells for control (2), standards (14) and test sample (All in duplicate)
2. Prepared the control (S_0) and working standards (S_1 to S_7) as per the kit instruction

a. Diluted the stock solution of standard with EIA buffer to obtain concentrations 0.04, 0.1, 0.2, 0.4, 1, 2 and 10 ng /ml being standards S_1 to S_7 respectively
b. EIA buffer as such was used for the control (S_0)

3. Diluted the cortisol enzyme conjugate 1:50 (110 µl in 5.5 ml) with EIA buffer

4. Added 50 µl each of the control, standards or sample in duplicate into appropriate wells
5. Added 50 µl of conjugate to each well, mixed and incubated at room temp for one hour
6. Diluted the concentrated wash buffer (20 ml in 180 ml) with distilled water
7. Washed the microplate five times with diluted wash buffer in an automatic washer
8. Added 150 µl of the substrate into the wells and incubated at room temp for 30 minutes
9. Added 100 µl of 1 N HCl into each well for stopping the chromogenic reation
10. Read the Optical density absorbance at 450 nm in the microplate reader
11. Mean of OD values for the duplicates were taken as B values for each well component.
12. Calculated the B/B_0x100 for each of the wells (where B_0 - OD value for the control)
13. A standard curve was plotted using concentration and $\%B/B_0$ values for the standards
14. Interpolated the cortisol value of each sample based on $\%B/B_0$ from the standard curve
15. Multiplied the obtained value with 100 to obtain the cortisol concentration in the serum

Appendix 7.

Procedure for MDA Estimation

Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measured at 535 nm. The procedure involved

Preparation ofTCA – TBA – HCl Reagent

- Dissolve 15% W/V trichloroacetic acid and 0.375% W/V thiobarbituric acid in 0.25N Hydrochloric acid (HCl)
    - Measured 2.1 ml of concentrated Hydrochloric acid (HCl) and mixed with distilled water until the volume reached 100 ml to make 0.25 N HCl
    - Dissolved 15 gm TCA and 0.375 gm thiobarbituric acid in the 0.25 N HCl and volume was made up to 100 ml.
    - Heated the mixture to achieve complete dissolution of TBA

MDA estimation

- Took 0.4 ml serum and added 0.6 ml TCA-TBA-HCl mixture
- Mixed well and kept in boiling water bath for 10 minutes
- Allowed the reaction mixture to cool down to room temperature
- Centrifuged for 5 min at 3000 rpm and collected the supernatant (Pink colour)
- Blank was prepared by replacing serum with distilled water
- Read the absorbance using spectrophotometer at 535 nm wave length
- Compared absorbance of the sample against that of the blank
- MDA concentration was calculated as optical density / 0.0624 µ.moles /ml

Calculation

Molar extinction coefficient of MDA at 535 nm is = 1.56×10^5 L/M concentration.

1.56×10^5 L contains = 1 M concentration

1 L contains = 1M / 1.56×10^5 concentration

We know that Density = Mass / Volume

Here Mass is = 1M / 1.56×10^5 and volume is 1 litre

OD = M/ 1.56×10^5, Litre = M/ 1.56×10^8 ml concentration

1 OD equal to = $1 / 1.56 \times 10^8$ ml concentration

χ is the optical density obtained, then

χ (OD) equal to = $\chi / 1.56 \times 10^8$ mole concentration

Here χ (OD) – represents 0.4 ml of serum

So 0.4 ml of serum contains MDA concentration = $\chi / 1.56 \times 10^8$ mole

1 ml of serum contains MDA concentration = $\chi / 1.56 \times 10^8 \times 0.4$ mole

MDA concentration = $\chi / 1.56 \times 10^8 \times 0.4$ mol / ml

Or = $\chi / 0.156 \times 10^9 \times 0.4$ mol / ml

Or = $\chi / 0.0624 \times 10^9$ mol / ml

Appendix 8.

ELISA for Oestrogen

. The ELISA kit (Calbiotech inc., El Cajon Canada) was used for Oestradiol assay. The kit works based on the principle of delayed competitive binding assay between estrogen in the sample and enzyme conjugate for a constant amount of anti Oestradiol monoclonal antibody epitop. The kit components included

- Microplate - 96 well plate coated with Streptavidin
- Oestradiol standards (6),
- Oestradiol biotin conjugate
- Oestradiol enzyme conjugate concentrate,
- Assay diluents
- TMB substrate
- Stop solution and
- Wash buffer concentrate.

In the incubation anti E2 antibody biotin reagent, E2 standards, controls and samples were incubated for 45 minutes at room temperature. E2 enzyme conjugate was added on the top of the reaction mixture and incubation continued for 45 minutes more. During the incubation a fixed amount of HRP labeled E2competes with estradiol in the sample / standard for a fixed amount of binding sites. Unbound reagent and conjugate were then removed by washing and conjugate bound onto the sites was estimated by the addition of TMB substrate through the colour development. Intensity of colour was measured spectrophotometrically at 450 nm. Standard curve was plotted using OD values for the standards and E2 concentration in the serum samples were interpolated based on the OD values for each of them.

Procedure

1. Prior to the assay, frozen serum samples were thawed and mixed well
2. Working solution of enzyme conjugate (1:20) was prepared by adding 0.6 ml of the concentrate to 11.4 ml of assay diluents)
3. Diluted the wash buffer (25 ml) with 475 ml of distilled water
4. Brought all the reagents to room temperature before use and mixed gently
5. Wells for standards, control and samples were identified

6. Took 25 µl of serum and standards (6) into the wells (in duplicates)
7. Added 50 µl working solution of oestradiol – biotin reagent into each well
8. Mixed and incubated at room temperature for 45 minutes
9. Add 100 µl of oestradiol enzyme conjugate to all the wells
10. Mixed well and incubated again at room temperature for 45 minutes
11. Removed the liquid and washed the wells three times in an automatic washer
12. TMB substrate (100 µl each) was added into all the wells
13. Mixed by shaking and incubated at room temp for 20 minutes
14. Added 50 µl of stop solution into the wells
15. Read the optical density (within 15 min) at 450 nm
16. Calculated the mean absorbance value for each set of standards and samples
17. Concentration of oestradiol was obtained from the Micro plate manager software

Appendix 9.

ELISA for Progesterone

The Pathozyme kit for progesterone (Omega diagnostics Ltd) works based on the principle of competitive binding between P4 in the test sample and HRP conjugated P4 for a constant amount of rabbit anti P4 antibody. The Kit was provided with the usual components such as

- Microplate - 96 well plate coated with goat anti rabbit IgG,
- Five standards,
- Two controls with known P4 concentration,
- Rabbit anti P4 reagent,
- HRP conjugated P4,
- Diluents for the conjugate,
- TMB substrate and stop solution,

In the assay procedure caprine derived anti rabbit IgG coated wells were incubated with P4 standards, controls, samples to be assayed, P4 HRP conjugate reagent and rabbit anti P4 reagent. After the incubation, unbound P4 conjugate was removed by washing and substrate was added to quantify the amount of bound P4 peroxidase enzyme through suitable colour development. The colour formation was stopped by the addition of stop solution and the colour intensity was measured spectro photometrically as optical density value at 450 nm. A standard curve was plotted using the OD values for the standards and P4 concentrations for the unknown samples were interpolated from the curve. The assay procedure sequence was as follows

1. Prior to assay, frozen serum were thawed and mixed well
2. Brought all the reagents to room temperature before use and mixed gently
3. P4 enzyme conjugate was diluted 1:10 (1.3 ml in 13 ml) with the diluent
4. Allocated the wells for standards, control and samples (in duplicates)
5. Added 25 µl of the standards (5), control (1) and samples into the wells
6. Working solution of enzyme conjugate (100 µl each) was added into all the wells
7. Added 50 µl of rabbit anti progesterone reagent to all the wells
8. Mixed well and incubated at room temperature (20-25 ºC) for 60 minutes
9. Removed all the liquid and washed the wells three times in automatic washer

10. Added 100 µl of TMB substrate to all the wells and incubated for 15 minutes
11. Added 50 µl of stop solution to all the wells and mixed by gentle shaking
12. Optical density was read within 15 minutes using microplate reader (450 nm)
13. OD values for the blank was subtracted from that of sample and the standards
14. Standard curve was plotted using OD values for the standards
15. Interpolated progesterone concentration of the samples were obtained from the Microplate manager software

Appendix - 10

List of abbreviations used

AI	- Artificial insemination
am	- Ante-meridiem (Fore-noon)
ANOVA	- Analysis of variance
AbT	- Ambient temperature
AtT	- Atmospheric temperature
AvRH	- Average relative humidity
AvT	- Average temperature
BCS	- Body condition score
BS	- Brown Swiss
BT	- Body temperature
CL	- Corpus luteum
CP	- Crude protein
CR	- Conception rate
ºC	- Degree centigrade
DAI	- Double artificial insemination during the oestrus
DJF	- December – January - February (Post monsoon)
DMI	- Dry matter intake
E2	- Oestrogen
EED	- Early embryonic death
ELISA	- Enzyme linked immune sorbent assay
FCL	- Functional corpus luteum
FSH	- Follicle stimulating hormone
°F	- Degree Fahrenheit
GnRH	- Gonadotropin releasing hormone
Gr.	- Group
h	- Hours
HF	- Holstein Fresian
HSP 70	- Heat shock protein – 70
ICAR	- Indian Council of Agriculture Research
IFN-τ	- Interferon-τ
IPCC	- Inter-continental panel on climate change

JJA - June-July-August (South West monsoon)
Kg - Kilogram
LH - Luteinizing hormone
MAM - March – April – May (Summer)
MDA - Malondialdehyde
mL - Milli-litre
MnRH - Minimum relative humidity
MnT - Minimum Temperature
mRNA - Messenger ribo-nucleic acid
MxRH - Maximum relative humidity
MxT - Maximum temperature
µ.moles - Micro moles
ng - Nano-gram
NIANP - National Institute of Animal Nutrition and Physiology
ODR - Oestrus detection rate
P4 - Progesterone
pg - Pico-gram
$PGF_2\alpha$ - Prostaglandin F_2 alpha
pm - Post-meridiem (After-noon)
PP - Post-partum
RCL - Regressing corpus luteum
RH - Relative humidity
ROS - Reactive oxygen species
RR - Respiratory rate
RT - Rectal temperature
SAI - Single artificial insemination during the oestrus
SE - Standard error
SON - September-October- November (North-east monsoon)
TDN - Total digestible nutrients
THI - Temperature humidity index
TS - Thermal stress
USG - Ultrasonography

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INFLUENCE OF THERMAL STRESS ON POST-PARTUM REPRODUCTIVE PERFORMANCE OF CROSSBRED DAIRY COWS

C. IBRAHEEM KUTTY

(2016-DVP-002)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

(Animal Reproduction, Gynaecology and Obstetrics)

2020

Faculty of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University

DEPARTMENT OF ANIMAL REPRODUCTION,
GYNAECOLOGY AND OBSTETRICS
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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KERALA, INDIA

ABSTRACT

Together with increasing productivity of dairy cows, vulnerability to thermal stress (TS) and fertility impairments have become serious concern in the context of ongoing climate change phenomenon. The study was carried out at LRS Thiruvazhamkunnu in Kerala, to elucidate TS influence on reproductive performance of crossbred dairy cows and to identify suitable biological markers for timely intervention. The study involved retrospective assessment of reproductive performance in comparison with weather parameters over a period of six years and prospective investigation of the climatic stress impact on various production and reproduction parameters round the year.

Major climatic stress factors showed significant ($P<0.001$) variation between seasons with highest ambient temperature during summer and lowest in rainy season. Relative humidity was rather high throughout the year so that THI was high enough to cause mild to moderate TS across all the seasons. Corresponding to the extended influence of TS factors, reproductive performance was much below the expected level in all the seasons.

Prospective study also showed almost similar pattern of stress impact throughout the year. The biological response assessed based on HSP 70 and Cortisol levels in the serum indicated the prevalence of TS during all the seasons with maximum during summer. Respiration rate, rectal temperature and serum MDA level also showed highly significant elevation during summer being the immediate response to stress. However, production and reproduction related parameters showed more or less uniform performance irrespective of the seasons.

It is concluded that overall lowered herd fertility is the consequence of TS exposure throughout the year. In spite of more adverse climate during summer, uniform fertility across seasons appears to be due to the adaptation mechanism, reflected by the high level of HSP 70 in the serum. Hence HSP 70 can be used as an effective indicator of TS in crossbred dairy cows.

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Academic Form No. 19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

FACULTY OF VETERINARY AND ANIMAL SCIENCES

PROGRAMME OF RESEARCH WORK FOR THESIS FOR DOCTORATE DEGREE

1. Title of Thesis

Influence of thermal stress on post-partum reproductive performance of crossbred dairy cows

2a. Title of the departmental/KVASU research project of which this form a part

Nil

2b. Code No. if any and order by which departmental/KVASU research project is approved

Nil

3a. Name of the student

C. Ibraheem Kutty

3b. Admission No.

2016-DVP-002

3c. Name of the discipline:

Animal Reproduction, Gynaecology and Obstetrics

4a. Name of Major advisor (Guide)

Dr. C. P. Abdul Azeez

4b. Designation

Assistant Professor,
Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences, Pookode, Wayanad-673 576

5. Objectives of the study

1. Elucidate the influence of thermal stress on post-partum reproductive performance of crossbred dairy cows
2. Identify suitable physiological indicators for assessing the magnitude of thermal stress on fertility of crossbred cows

6. Practical/Scientific utility

Reproductive rate is one of the major determinants of profitability from any type of animal production enterprise. Consequent to genetic alterations and improvement of management standards, considerable increase in milk production of dairy cows has been recorded worldwide. At the same time, fertility of these animals started to decline, marginalizing the benefits of hike in milk production. Various forms of stress, which interfere with hormonal milieu of the animal have been attributed for the decline in fertility.

Climate change is becoming one of the major threats for the enhancement of animal productivity especially at the tropical regions with high ambient temperature. Hot humid climate prevailing in Kerala has already been found hostile to productivity of crossbred cattle. Inconsistency and extremes of ambient temperature and humidity are going to affect the productivity of crossbred cows and subsequent fertility decline is inevitable.

Various management measures for the alleviation of environmental stress factors have been found to reduce the adverse influences of stress on milk production and metabolic parameters, with little benefits for restoration of fertility. However, indicators for an imminent fertility reduction consequent to thermal stress (TS) and the stage to intervene for prevention of the same are yet to be established. Hence, there is a need for precise understanding of the stress mediated fertility impairments and identification of suitable physiological indicators so that necessary stress alleviation measures can be adopted for minimizing the impact of climate change on fertility and productivity of animals.

7. Important publications on which the study is based

Thermal stress reduced the degree of dominance of the selected follicle which was manifested by reduced steroidogenic capacity of theca and granulosa cells. Follicular activity, ovulatory mechanism, oocyte and embryo quality and uterine environment were altered by the endocrine changes consequent to TS, reducing the likelihood of embryo implantation (De-Rensis and Scaramuzzi, 2003).

Exposure of Gir cows to TS had no immediate effect on reproductive function, however, it exerted a delayed deleterious effect that lasted up to 105 days on ovarian follicular growth, hormone concentrations and oocyte competence (Torres *et al.*, 2008).

Boni *et al.* (2014) observed significant reduction in conception rate (CR) of cows during summer months with higher temperature humidity index (THI) and it was attributed to reduced oestrus detection consequent to TS.

Min *et al.* (2015) found that serum levels of heat shock transcription factor and heat shock protein (HSP) 70 were sensitive and accurate indicators of TS and recommended these factors as potential biomarkers to supplement THI and evaluate moderate TS in dairy cows.

In vitro studies by Pavani *et al.* (2015), subjecting oocytes and embryos to TS, showed significant decline in nuclear maturation rate of oocytes, cleavage rate and development of embryos with every 1°C rise in temperature during summer and winter months. *In vivo* studies in grazing cattle also showed a negative correlation between CR and THI values.

Schuller *et al.* (2015) noticed that exposure of lactating dairy cows to TS with mean THI of 73 or more for a short duration significantly reduced the CR. The adverse effect was observed both before and after the day of breeding with the greatest impact being from day 21 to the previous day of oestrus.

Dash *et al.* (2015) observed a THI of 75 as the threshold level for onset of TS affecting pregnancy rate. May and June months were identified as the critical heat stress zone with a maximum decline of seven per cent pregnancy rate with every unit increase in THI.

Paul (2016) studied the effect of TS on fertility of crossbred cattle in Kerala during the two seasons with highest and lowest THI values. They found no significant variations in fertility related parameters such as intensity and duration of oestrus, rheological properties of cervical mucus and serum progesterone levels. However, CR was found to be better during the phase of low THI.

Zarina (2016) found that cortisol levels remained consistent irrespective of the THI values, where as HSP 90 expression showed significant ($P<0.05$) up regulation from THI 82.0 onwards in cattle and buffalo calves maintained at central midlands of Kerala.

De-Rensis *et al.* (2017) reported a direct effect of TS on hypothalamo-pituitary-ovarian axis and on the secretion of cortisol and prolactin during summer. They also observed an extended oestrus to ovulation interval with modified gonadotrophin secretion which lead to high incidence of anovulatory follicles, ovarian cysts, inferior quality oocytes and luteal dysfunction thereby increasing the risk of pregnancy loss.

Schuller *et al.* (2017) reported significant reduction in the intensity of oestrus signs and the size of follicles with increased THI.

8. Outline of the technical programme

The study will be conducted at Livestock Research Station, Thiruvazhamkunnu, under KVASU. The study will be carried out in three parts.

Part I: Retrospective assessment of the reproductive performance and periods of thermal stress

Breeding records of the farm will be screened for retrospective assessment of reproductive performance of the herd during previous five years. Animals affected with Tuberculosis or Brucellosis will be excluded from the study. Past recordings of meteorological data of the locality will be collected and the study period will be divided based on high and low THI demarcated by the zone of thermal comfort. The fertility parameters will be then compared with the

THI of corresponding periods and also between seasons to appreciate the influence of thermal stress factors

Part II: Prospective assessment of the influence of thermal stress on post-partum fertility

Apparently healthy cows (n=20) of second to fifth parity, having almost similar milk yield, body condition score (BCS) and without a history of recent peri-partum complications, will be selected for the study from Day 28 to 90 PP. Cows will be maintained under identical conditions of housing, feeding and general care and the following parameters will be recorded

Climatic variables

Climatic variables namely, daily maximum - minimum temperatures and relative humidity will be recorded throughout the period of study and will be utilized for arriving periods of high and low THI.

Production parameters

Milk production and feeding details of the selected animals will be recorded daily throughout the period of study.

Body condition score

Body condition score of cows under the investigation will be recorded at seven days interval throughout the period of study as suggested for crossbred cows by Smijisha (2012).

Oestrus characteristics

Cows will be regularly watched for signs of oestrus at morning and evening starting from Day 28 PP. Intensity and duration of observed oestrus will be assessed using subjective scores as described by Van-Eerdenburg *et al.* (1996).

AI and pregnancy diagnosis (PD)

Cows in proper stage of oestrus will be inseminated using cryopreserved semen and observed for return to oestrus up to Day 25 post AI. Details of AI and inter-oestrus interval in return cases will be recorded. Conception rate will be assessed based on PD at Day 45, using B mode transrectal ultrasound scanning, and confirmation at 90 days post AI by palpation per rectum.

Eight out of the 20 PP cows will be randomly selected for detailed investigation. The following parameters will be studied at seven days interval, throughout the study period starting from Day 28 PP.

a. Resumption of PP cyclical activity: Cows will be routinely observed and subjected to clinico-gynecological examination at seven days intervals and cows detected in oestrus will be inseminated.

b. Growth of ovarian follicles: B-mode trans-rectal ultrasound scanning of both the ovaries will be done in order to assess the number of follicles of ≥ 8 mm diameter, having potential to become dominant (Ginther *et al.* 1996).

c.Progesterone level: Blood samples will be collected for progesterone estimation using Enzyme immune assay (EIA) kits, at seven days interval from Day 28 PP until detection of oestrus /AI (day 0) and thereafter on 7, 14 and 21 days post AI. Progesterone level and functional status of corpus luteum during the cycle will be studied.

d. Oestrogen level: Blood samples will be collected on day 0 and day 1 of oestrus detection for the estimation of oestradiol level (by EIA Kits) to study the variations in cyclical peak of oestrogen.

Inseminated cows will be exempted from clinico-gynaecological examination and ultrasound scanning up to 25 days post AI and those returned to oestrus will be re-enrolled for clinical examination, scanning and blood collection.

e. **Pregnancy loss:** Inseminated cows not returned to oestrus will be subjected to PD by B-mode transrectal ultrasound examination on Day 25 post AI and thereafter on days 35, 45, 60 and 90 post AI to detect pregnancy loss.

f. **BT and respiration rate:** BT and respiration rate of the selected animals will be recorded at seven days interval during the periods of high and low THI and the observations will be utilized to compare the variations.

g. **Thermal stress indicators:** Bloodsamples will be collected at seven days interval during the period of high THI and an equal number of samples during low THI period for monitoring thermal stress associated factors namely cortisol and HSP 70 (using standard EIA kit).

h. Antioxidant levels in serum: Bloodsamples will be collected at seven days interval during the period of maximum THI for detection of malondialdehyde (MDA) levels (using spectrophotometry) to understand the antioxidant status during thermal stress.

Part III. Screening of collected samples and data analysis

Blood samples collected will be subjected to evaluation of different parameters as mentioned. The data collected will be statistically analyzed as per Snedecor and Cochran (1994). Grouping of the animals as thermal stress affected and unaffected will be carried out based on the HSP 70 values assessed during the period of study. The observations on climatic variables, otherstress indicators (cortisol and MDA levels), fertilityparameters, conception details, hormone levels and follicular emergence will be compared between the two groups as well as seasons to assess the association of thermal stress with climatic and fertility parameters.

Table1. Summary of study parameters

Study parameters	***Data collection***
Fertility parameters in the past five years	Farm records
Climatic variables in the past five years	Weather station data
Climatic variables during the study period	Daily recording
Details of milk production and feeding (20 cows)	Daily observation
BCS (n=20)	7 days interval
Oestrus characteristics (n=20)	Routine
AI and PD (among 45 day non-returns), (n=20)	Timely
Resumption of PP cycle – clinical examination (n=8)	7 days interval from 28 days PP
Emergence of follicles – by ultrasonography (n=8)	7 days interval from 28 days PP
Serum progesterone level (n=8)	7 days interval from 28 days PP
Progesterone level during oestrous cycle (n=8)	Days 0,7,14 and 21 post oestrus
Oestrogen level during oestrus (n=8)	Days 0 and 1 of oestrus
Pregnancy loss among conceived by ultrasonography	Days 25, 35, 45, 60 & 90 Post AI
BT and respiratory rate (n=8)	7 days interval
Blood sampling for HS markers and MDA (n=8)	7 days interval during HS

9. Main items of observations to be made

A. Retrospective study

- Reproductive performance of cows during the past five years
    - number of breedable cows
    - cows observed to be in oestrus
    - number of inseminations
    - number of cows conceived
    - embryonic / foetal loss or abortions
    - number of calvings per month
- Meteorological data for past five years

B. Prospective study

- Daily recording of climatic variables
- Details of feeding and milk yield
- Body condition score
- Oestrus characteristics
- Details of cows inseminated
    - One to three AI
    - Repeat breeder cows (> 3 AI)
- Conception and pregnancy loss
    - non-return cows at Day 25 post AI
    - pregnancies at Day 25, 35, 45, 60 and 90
    - cows suffered embryonic loss / early abortions
- BT and respiratory rate

- Number of follicles of size ≥ 8 mm
- Serum progesterone level (ng / ml)
- Serum oestrogen level (pg/ml)
- Estimation of serum cortisol (µg/dl)
- Presence / level of HSP 70 in serum (ng/ml)
- MDA level (µmol / ml)

10. Facilities

(a) Existing:

Facilities available at Livestock Research Station, Thiruvazhamkunnu and the Department of Animal Re production, Gynaecology and Obstetrics, CV&AS, Pookode; and Central Instrumentation Lab of the College of Veterinary and Animal Sciences, Pookode and Mannuthy will be utilized.

(b)Additional facilities required:

Data from Meteorological stations of CAADECCS at Thiruvazhamkunnu and Mannuthy.

11. Duration of study

Six semesters

12. Financial estimate

Cost of lab wares, assay kits, medicines, disposables etc.	:	Rs. 45,000
Contingencies	:	Rs. 5,000
Total	:	Rs. 50,000

Signature of the Student

Signature of the Major Advisor

Place: Pookode

Date: 30/06/2018

Name, Designation and Signature of the members of the Advisory Committee:

Chairman

Dr. Abdul Azeez C. P.

Assistant Professor
Department of Animal Reproduction,
Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences,
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Members

1. Dr. Promod K.

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APPENDIX - I

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APPENDIX – II

Time frame of work

Semester I

1. Collection of literature
2. Planning programme of research work
3. Preparation of synopsis

Semester II

1. Collection of literature
2. Purchase of essential items
3. Preparatory steps for data collection
4. Preliminary data collection

Semester III

1. Collection of literature
2. Recording climatic variables
3. Collection of retrospective data
4. Selection and grouping of animals
5. Recording of feeding and milking
6. Animal examination / scanning
7. Sample collection and storage
8. Breeding and follow up

Semester IV

1. Routine grouping of animals
2. Recording of feeding and milking
3. Animal examination / scanning
4. Sample collection, storage and screening
5. Breeding and follow up
6. Follow up studies

Semester V

1. Sample collection, storage and screening
2. Follow up studies of breeding

Semester VI

1. Analysis of data and interpretation
2. Preparation of thesis and publication

CURRICULUM VITAE

1.	Name	Dr. C. IBRAHEEM KUTTY
2.	Date of birth	25th May 1969
3.	Place of birth	Mattathur – Malappuram District, Kerala
4.	Marital status	Married
5.	Permanent address	Cholakkal House, Mattathur P.O. 676528
6.	Major field of specialization	Animal Reproduction, and Public health
7.	Educational status	Master Veterinary Science (Animal Reproduction) Kerala Agricultural University - 1995 Master of Public Health -Sree Chitra Thirunal Institute forMedical Science and Technology – Trivandrum (Deemed University) - 1999

8. Professional experience

Twenty eight years long career in Veterinary field under government / University / semi government and private institutions in India and abroad. Major activities included Veterinary Services, Animal fertility enhancement, Farm management, Animal breeding activities, Professional teaching, Animal reproduction and public health research, Technical publication and extension activities. Experience of working with different species including Livestock, camels, equines, pets and poultry, commercial dairy farms, mixed game farm, veterinary clinics, teaching institutions and research centres in UAE, Saudi Arabia, and India.

9.	Publications	Many scientific and popular publications	
		Text bookspublished	4
		Chapters in reference books / compendium	2
		Research articles international journals	2
		Research articles in Indian journals	25
		Technical articles in English	15
		Technical articles in Malayalam	30

Dr. C. Ibraheem Kutty