EFFECT OF HEAT STRESS ON THE EXPRESSION PATTERNS OF DIFFERENT REPRODUCTION RELATED GENES IN MALABARI GOATS

by

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(2013-20-107)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

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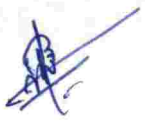
DECLARATION

I, hereby declare that this thesis entitled **"Effect of Heat Stress on the Expression Patterns of different Reproduction related Genes in Malabari Goats"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



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Amitha J Pai

Dedicated to Sejian six

and

My Family

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SYMBOLS AND ABRREVIATIONS

μL- Microliter

μM- Micrometre

ACTH- Adrenocorticotropic hormone

ADG- Average daily gain

ANOVA- Analysis of variance

AT- Ambient temperature

BCS- Body condition scoring

bp- Base pair

BW- body weight

°C- Degree Celcius

cDNA- Complementary DNA

CO_2- Carbon dioxide

COX-2- Cyclooxygenase-2

CRH- Corticotropin releasing hormone

DMI- Dry matter intake

DNA- Deoxy ribonucleic acid

EDTA- Ethylenediaminetetraacetic acid

ESTRα- Estrogen Receptor α

FAO- Food and Agriculture Organization

FSH- Follicle Stimulating Hormone

FSHR- Follicle stimulating Hormone Receptor

GAPDH- Glyceraldehyde 3-phosphate dehydrogenase

GH- Growth hormone

GHGs- Greenhouse gases

HPA- Hypothalamo-Pituitary-Adrenal axis

HPG- Hypothalamo-Pituitary-Gonadal axis

IGF-1- Insulin like Growth Factor

IPCC- Intergovernmental Panel on Climate Change

LH- Luteinizing Hormone

LHR- Luteinizing hormone receptor

m- Metre

MC- Malabari Control

Mg- Milligram

MHS- Malabari Heat Stress

mm- Millimetre

NCBI- National Centre for Biotechnology Information

ND- Nano Drop

Ng/ml- Nanogram/milliliter

PCR- Polymerase Chain Reaction

PGE2- Prostaglandin E2

PGF2α- Prostaglandin F2 α

qPCR- quantitative polymerase chain reaction

RH- Relative Humidity

RNA- Ribonucleic acid

RNase- Ribonuclease

rpm- Revolutions per minute

RTqPCR- real-time quantitative polymerase chain reaction

SNF- Solid not fat

THI- Temperature Humidity Index

TNZ- Thermoneutral zone

USA- United States of America

INTRODUCTION

CHAPTER 1

INTRODUCTION

The livestock sector plays a crucial role for the livelihood security of the farming community. The livestock acts as the source of revenue for 1.3 billion poor people (FAO, 2009). The farming community primarily relies on the productive parameters and reproductive performance of the livestock for their livelihood. Currently, climate change was considered as the most threatening factor affecting the welfare of the livestock. Among all the climatic variables, heat stress can be considered as the most detrimental factor to the livestock population. The severity of heat stress is multifold when the elevated ambient temperature is coupled with high relative humidity. Therefore, heat stress affects all the productive parameters of the animal and concomitantly threatens their survivability. The animals in tropics and sub tropics are under severe heat stress (Salles *et al.,* 2010). Although the animals possess the capabilities to adapt to the changing climate, they do so by compromising their productive functions especially the reproduction so as to deviate energy resources for maintaining the life sustaining activities (Sejian *et al.,* 2011).

Among all the livestock species, small ruminants play a vital role in securing the livelihood of rural community as they offer meat, milk, offal and wool (Salem, 2010). Further as compared to large ruminants, small ruminants and goats in particular are considered to be more resilient to the changing climatic condition. Goats possess the higher resilience capacity than cattle and sheep due to their higher thermo-tolerance, drought tolerance, ability to survive on limited pastures and highly disease resistance capability (Silanikove, 2000; Jakper and Kojo, 2014). Further, goats possess higher feed conversion efficiency and have the better ability than other ruminat species to efficiently convert the feed resources into either meat or milk (Ayele *et al.,* 2008; Hailu 2014). In addition, goat also has the ability to

thrive well in harsh environmental conditions, which paves the way for the scientific community to earmark goat as the ideal animal model for climate change. Moreover, indigenous goats were found to be more adapted than the crossbred or pure bred animals (Wheelock *et al.,* 2010). Therefore, it is very vital to study the adaptive capacity of local goat breeds in an effort to identify the most suitable breed for a specific location.

The impact of heat stress on reproduction has been widely established cutting across species particularly in dairy cattle (Garcia-Ispierto *et al.,* 2007), buffaloes (Dash *et al.,* 2015), sheep (Marai *et al.,* 2000) and goat (Ozawa *et al.,* 2005). The study undertaken by Gwazdauskas (1985) clearly emphasized that the heat stress imparted a detrimental effect on livestock, reducing the conception rate by 20 percent to 27 percent. Similarly, the elevated temperature affects the secretion of gonadotropin which leads to inadequate production of estrogen and progesterone (Scholtz *et al.,* 2013). Several studies have stressed the adverse impacts of heat stress on the endometrial functions and secretary activities in various livestock species (Wolfenson *et al.,* 2000). Thus, the imbalance in secretions and low progesterone level (Lamming and Royal, 2001) could lead to early expression of luteolytic mechanism and further fails in implantation (Khodaei *et al.,* 2011). Similarly, heat stress condition induces significant changes in the production of ACTH and prostaglandin F2 alpha (PGF2 α). Further, heat stress also was established to negatively influence the follicular dynamics leading to compromised reproductive performance (Wolfenson *et al.,* 2000).

Although, several studies established the impact of heat stress on reproduction based on changes in the phenotypic traits, researches on the implications of the same based on genotypic traits are very scarce. In addition, the underlying molecular mechanisms by which heat stress induced changes in the genetic traits pertaining to animal reproduction are not elucidated. Hence it is high

time that such research efforts are oriented towards establishing the basic molecular mechanism of heat stress induced changes in animal reproduction.

Therefore, this study attempts to reveal the hidden intricacies associated with the heat stress induced changes in the reproductive traits of indigenous Malabari goat breed. The study was conducted with the primary objective of establishing the effect of heat stress on expression patters of different traits that controls Malabari goat reproduction. The study may yield suitable genetic markers governing reproduction during heat stress exposure. Identification of such markers through genetic selection and incorporating those genes in thermo-sensitive breeds may increase the possibilities of higher productivity in heat stressed animals.

The objectives of the study are:

1. To assess the expression patterns of follicle stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) gene expression in Malabari goats subjected to heat stress.

2. To evaluate the expression patterns of prostaglandin and estrogen receptor genes in heat stressed Malabari goats.

3. To establish the correlation between THI and expression patterns of different reproduction related genes in heat stressed Malabari goats.

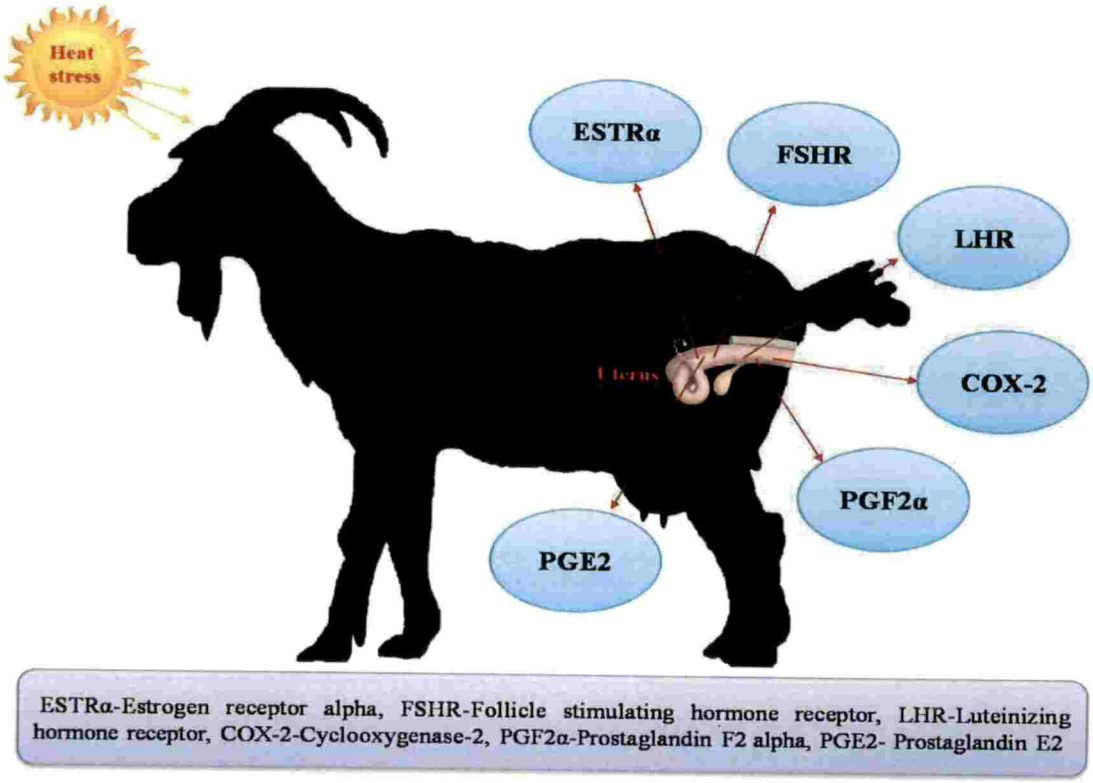


Fig.1.1: Hypothetical figure describing the expression pattern of different reproduction related genes during the heat stress exposure in goat

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

General Overview

The performance, health, and well-being of livestock are strongly affected by climate. High ambient temperatures, high direct and indirect solar radiation and humidity are environmental stressing factors that impose strain on animals. Among the environmental variables affecting livestock, heat stress seems to be one of the most intriguing factors hampering animal production in many regions of the world (Sejian *et al.*, 2011). Even though new knowledge on the animal responses to the environment continually arises, managing livestock to reduce the impact of climate remains a challenge. Considerable efforts are therefore, needed from livestock researchers to counter the impact of environmental stresses on livestock production (Sejian *et al.*, 2010). Besides ensuring the livelihood security to our poor and marginal farmers, stress mitigation can also improve the economy of livestock industry as a whole. Hence, it is crucial to understand the impact of environmental stress on livestock production and reproduction. These efforts may help in identifying the appropriate targets for developing suitable mitigation strategies.

Thermal stress effects on livestock are of multifactorial nature. It directly alters and impairs the cellular functions in various tissues of body and the redistribution of blood flow, as well as the reduction in food intake, which ultimately results in reduced production performance (Bagath *et al.*, 2016). Reproductive functions of livestock are particularly vulnerable to climate change; it has been established that large ruminants are more prone to heat stress compared with small ruminants (Naqvi *et al.*, 2012). Heat stress is the major cause for infertility and reproductive inefficiency in livestock, resulting in profound economic losses (Maurya *et al.*, 2016). Heat stress reduces the libido, fertility and embryonic survival in livestock, and favours the occurrence of diseases in neonates with reduced

immunity (Schuller *et al.*, 2014). Heat stress affects the fertility and reproductive performance of livestock species through compromising the functions of the reproductive tract, disrupting the hormonal balance, decreasing the oocyte quality, and thereby decreasing embryo development and survival (Gendelman and Roth, 2012a). In the tropical and subtropical regions, during the hot season, both the poor-quality of oocytes and embryos resulted in decreased conception rate and subsequently with more days open resulting in huge economic losses to the dairy industry (Kumar *et al.*, 2017). The high ambient temperature and relative humidity directly affected reproduction by altering or impairing various tissues or organs of the reproductive system of animal (Naqvi *et al.*, 2012). The threshold level of temperature humidity index (THI) for the high performance in terms of milk yield and reproduction is around THI 72 in tropical and subtropical climates.

High environmental temperatures impaired the female reproductive process at various stages of pubertal development, conception and embryonic mortality (Stewart *et al.*, 2011). Stress inhibits the reproductive performance of livestock species by activating the hypothalamic-pituitary-adrenal (HPA) axis, which subsequently excited the pituitary gland to release adrenocorticotropic hormone (ACTH). The ACTH stimulates the release of glucocorticoids and catecholamines, which act extensively to alleviate the effect of stress. However, ACTH stimulated glucocorticoid release is responsible for an inhibitory effect on the reproductive axis. Heat stress reduced the length and intensity of estrus, altered follicular development and increased the rate of apoptosis in the antral and pre-antral follicles (Ozawa *et al.*, 2005). Extreme environmental temperatures delay the onset of puberty in male and female animals. Furthermore, heat stress during follicular recruitment suppressed the subsequent growth and development to ovulation (Wolfenson *et al.* 2000). Changes in the follicular growth disturbed the further progress and function of oocytes (Naqvi *et al.*, 2012). The chronic release of ACTH associated with heat stress, inhibited the ovulation and follicular development by altering the efficiency of follicular selection

and dominance. The glucocorticoids were critical to mediate this inhibitory effect on reproduction (Sejian *et al.*, 2011). Further, high level of glucocorticoids during heat stress directly inhibited the meiotic maturation of oocytes. In addition, corticotropic releasing hormone (CRH) inhibited the ovarian steroidogenesis, derived of the decrease in the secretion of luteinizing hormone (LH). The consequent decrease in estradiol resulted in reduced length and intensity of estrus expression (Wang *et al.*, 2013).

2.1 Climate change global scenario

The temperature rise in the climate system was unequivocal, which clearly indicated the role of human intervention in climate change through the emission of various greenhouse gases (GHGs) especially carbondioxide (CO_2). As per the fifth assessment report of Intergovernmental Panel on Climate Change (IPCC), half of the current anthropogenic gas emissions in the atmosphere occurred during the time period between 1750 and 2011. Similarly, different climate model projected that more than half of the global average temperature increased during the period 1950 to 2010, exclusively due to the GHG emission along with the associated anthropogenic forcing. Further, it had been projected that if the GHG emission continues in the current rate the global temperature is likely to rise by 2.6-4.8°C by 2100 (IPCC, 2013).

The ocean acts as a sink for about 30 percent of the total CO_2 emitted to the atmosphere which resulted in ocean acidification reducing the ocean pH by 26 percent. Additionally, surface ocean layer warmed by 0.11 °C between 1971-2010. In addition, higher elevated atmospheric temperature bleaches the coral reef, which is a major habitat of marine ecosystem.

Moreover, melting of permafrost has become a prominent issue. Permafrost degradation has been reported from several parts of the world (Field *et al.*, 2014).

Further, reports of IPCC (2013) states that the thawing of permafrost and glaciers has raised the mean sea level by 0.17m – 0.21m during the time period 1901 to 2010.

The climate change resulted in an increased frequency and intensity of extreme events such as floods, tropical cyclone, heavy precipitations, drought, wild fires and heat waves (IPCC, 2013). Such extreme events imparted a severe effect on health, lives and associated environment and economic impacts. Moreover, erratic rainfall results in flooding in some parts of the world while the other regions suffer from severe drought. The alteration in the distribution of several vectors resulted in the emergence of new strains of vectors which risked the survivability of different species. In addition, the elevated temperature potentially increased the rate of extinction of different species and habitats. Climate change induced alteration in the lifecycle and development of several microbes intimidated the health as well as food safety (Hammond *et al.*, 2015).

2.2 Climate change and animal agriculture

The rapidly changing climate across the globe poses severe threat to the animal agriculture (Bernabucci *et al.*, 2010). The detrimental effect of climate change influenced the productive and reproductive performance and other welfare aspects of livestock. Climate change has both direct and indirect impact on livestock. The direct effects are by far mediated by increased ambient temperature which ultimately causes severe heat stress which jeopardizes the productive potential of animals. The indirect impacts are contributed by pasture and water non-availability and sudden outbreak of diseases (Nardone *et al.*, 2010). Studies established that climate change had a greater influence on the water availability, quality and quantity of pastures, and disease occurrence in farm animals (Thornton and Herrero, 2015; Rojas-Downing *et al.*, 2017). Variability in precipitation, temperature and atmospheric CO_2 levels influences the quantity and quality of forage crops (Rojas-Downing *et al.*, 2017). Further, in extensive animal production

systems, the animals has to move longer distances in search of feed and water due to shortage of natural resources which imparts locomotory stress to the animals (Sejian et al., 2018). The changing climate also predisposes several emerging diseases and parasitic challenges. Most of the production loses in farm animals arise as a result of aforementioned indirect effects of climate change (Koluman *et al.*, 2017).

Heat stress when coupled with high humidity aggravates the condition that ultimately impinge the growth rate, milk production, wool production and reproduction which affects food security and economy of rural society (Rust, 2013). Extreme weather influenced the growth performance of the animal affecting the feed intake, feed conversion potential and weight gain. As growth is a critical aspect for the meat production, diminished growth intimidated the meat industry particularly in the tropical region (Mpofu *et al.*, 2017). High ambient temperature mediated changes in the energy metabolism mechanisms which in turn affected the carcass quality (Pearce *et al.*, 2013). Further, reduced feed intake and exposure to testing conditions inordinately affected the reproductive functions of both male and female animals. These alterations involve reduced fertility, competency of oocyte, functional changes of ovary, embryonic development, embryo mortality (Naqvi *et al.*, 2012; Lacerda and Loureiro, 2015), estrus activity, pregnancy rate, declined sperm motility, dysfunctional sperm, sperm mortality (Silva *et al.*, 2015) and competence of development to blastocyst stage (Gendelman and Roth, 2012b). Pragna *et al.* (2017) emphasized that both milk yield and milk quality are affected simultaneously under hot and humid conditions as a result of adaptation process. In addition, it has been reported that temperature humidity index (THI) value beyond 72 appreciably reduced the percentage of milk fat, casein, lactalbumin, immunoglobulin G and immunoglobulin A in milk (Das *et al.*, 2016).

2.3 Heat stress as a major factor influencing livestock production

It is essential for every animal in any production system to be maintained in their thermal comfort zone (TNZ) for better performance. However, this comfort zone varies with species, breeds and individual animal. It comprises of an upper and lower critical temperature indicating their maximum threshold level of withstanding a stressed condition (Lees, 2016). Knowledge on these critical temperatures and comfort zone are very essential to provide ideal farm conditions for the livestock to sustain their production. As a consequence of climate change, heat stress affected the production performance of livestock resulted in adverse effect on the profitability of livestock enterprise. In Indian subcontinent, heat stress is the major climatic stress severely hampering the livestock productive capability and even their survivability (Sejian *et al.*, 2012). Hot environment weakened the metabolic and health status (immune response) of livestock. High ambient temperature (AT), relative humidity (RH) and radiant energy compromised the ability of animal to dissipate excess heat from the body. Heat is produced within the body as a result of physical activity of skeletal muscles and body tissue. This heat accompanied with a higher environmental temperature has a huge influence on the animal production performance. Hence, heat stress can be described as an outcome of the animal's inability to dissipate the excess heat load when they fails to maintain homeothermy which in turn leads to an elevated body temperature that triggers the initiation of several adaptive mechanisms to re-establish the homeostasis.

The additional heat gain during heat stressed condition alters the physiological and behavioral responses which negatively affected the productive and reproductive performances (Nardone *et al.*, 2010; Chauhan and Ghosh, 2014). Heat stress influences the hormonal changes which plays a major role in productive characteristics of farm animals (Bernabucci *et al.*, 2010). The activities of animal reach a resting level when the metabolic function reduces to decrease further heat production in order to maintain homeostasis. The maintenance energy expenditure

for cows above their thermoneutral zone increases by 20 percent especially when the temperature rise is above 35°C (NRC, 1981).

The concept of temperature humidity index (THI) has been used for predicting the stress level in animals. Among all the climate variables, maximum temperature and RH are the most critical variables to quantify heat stress (Dalcin *et al.*, 2016). The THI value above a threshold level of 72 indicates the animals are under severe stress and producers should adopt precautionary measures in anticipation of production loss beyond this range of THI (Brown *et al.*, 2016). The susceptibility of livestock to heat stress depends on the various factors such as diversity in genetic potential, species, life stages and nutritional status. A higher temperature in high latitudes imparted a greater impact on livestock population than those in the lower latitude where they are renowned for their adaptive capabilities to testing conditions (Thornton *et al.*, 2009).

2.3.1 Growth and meat production

Heat stress has a significant impact on the animal growth performance particularly in tropics and subtropics (Slimen *et al.*, 2015). Chronic heat stress reduced the circulating levels of growth hormone (GH) and it was also partially attributed to the plane of nutrition (Rhoads *et al.*, 2009). Extreme temperature affected the growth performance of animals by altering their feed intake, feed conversion efficiency, average daily gain (ADG), body weight (BW), body mass index (BMI), body condition scoring (BCS) and other allometric measurements (Sejian *et al.*, 2010; Niyas *et al.*, 2015). The adverse consequences of heat stress on growth includes reduced body weight gain, carcass yield, carcass protein content, muscle calorie weight and high mortality rates (Mujahid., 2011). As per Scientific Committee on Animal Health and Animal Welfare (SCAHAW, 2001), the maximum threshold temperature for a beef cattle is 30°C and RH below 80 percent. Therefore, temperature exceeding 30°C has a substantial influence on daily weight gain and

growth. Nardone (2000) reported reduced body size of cattle, sheep and goat during the dry months in Mediterranean area.

Heat stress has a prominent effect on meat production (Nardone *et al.,* 2010). Even though there are only limited literatures on impact of heat stress on meat, the reports emphasizes that the heat stress alters meat yield, meat quality as well as meat composition in livestock (Kadim, 2008; Gregory, 2010; Archana *et al.*, 2018). Declined feed intake in pigs due to heat stress leads to lean tissue growth which threatened the meat industry (Rauw *et al.*, 2017). The beef meat quality diminished when the average temperature increases to 34.3°C (Kadim *et al.*, 2004). The higher temperature also increased the loss of moisture that resulted in reduced tenderness and eating quality of meat (Nikbin *et al.*, 2016). Further, heat stress also drastically affected the muscle pH which imparted remarkable changes in the physicochemical attributes such as cooking loss, water holding capacity, meat colour and shear force (Gregory, 2010). Similarly in heat stressed broilers, Lu *et al.* (2017) observed a decrease in pH and simultaneously an increase in drip loss and sheer force of breast muscles.

2.3.2 Reproduction

The impact of heat stress on the reproductive performance was reported in cattle (Diaz *et al.*, 2018) and buffaloes (Ram *et al.*, 2017). Exposure of animals to heat stress affected the fertility of the animal. Heat stress reduced the fertility in dairy cattle by affecting the oocyte and embryo development (Lacerda and Loureiro, 2015). The oocytes subjected to heat stress lose their compatibility for fertilization and also failed in developing to blastocyst stage (Gendelman and Roth, 2012a and Gendelman and Roth, 2012b).

2.3.3 Milk Production

Lactating cattle are vulnerable to environmental temperature changes. Milk synthesis substantially decreases in the heat stressed dairy cattle. Cows are the most susceptible group of livestock due to larger body surface and prolonged exposure to high temperature especially during early lactation hampers the milk production. Cattle exposed to a temperature higher than 40°C drops the feed intake by as much as 40 percent (Rhoads *et al.*, 2013). It has been reported that the milk yield declined due to the cumulative effect of heat stress on feed intake, metabolic and physiological activities in dairy cattle (Najar *et al.*, 2010). Farm animals with high production potential are more susceptible to heavy heat loads as compared to the animals with lower production potential (Najar *et al.*, 2010). Generally in high producing cows at their peak lactation potential, the feed intakes as well as their metabolic rates were as higher as 2-4 times than at the maintenance state (Balamurugan *et al.*, 2018). Heat stress caused a response of rostral cooling center of hypothalamus which in turn stimulated the medial satiety center which restricted the appetite cooling center narrowed down to an immediate response of reduced feed intake and subsequently the milk production (Albright and Allitson, 1972). Heat stress modifies lipid metabolism as well as carbohydrate homeostasis in dairy cattle that alters the metabolic milieu which leads to severe production losses (Wheelock *et al.*, 2010). Kadzere *et al.* (2002) observed that when an animal is heat stressed a substantial quantity of water is lost through evaporation which may exceed the amount of water that is utilized for milk production. Hence, the water requirements during hot days are more in dairy cows.

In addition to milk yield, elevated core body temperature also significantly reduced the milk output, percentages of milk protein, fats, solids and lactose (Key *et al.*, 2014). Further the percentage of milk fat, solids-not-fat and milk protein reduced by 39.7, 18.9 and 16.9 percent in dairy animals as a consequence of continuous exposure to extreme ambient temperature (Kadzere *et al.*, 2002). Additionally, solid

not fat (SNF) and total solid percentage declined in the milk of animals under heat stress (Ozrenk and Inci 2008). Similarly, cows produce milk and colostrum with low fat and protein percentage when they are subjected to severe heat stress conditions (Nardone *et al.*, 1997). Further, higher ambient temperature was found to increase the udder temperature resulting in mastitis (Igono *et al.*, 1988). It has also been established that high producing genotypes are the most susceptible to heat stress effects in comparison with the well adapted indigenous breeds (Padodara and Jacob, 2013).

2.4 Economic consequences of heat stress impact on livestock production

The demand for protein and livestock products increases with an increase in global per capita income. The models predicted that dairy cattle are the most vulnerable group to the heat stress challenges in coming decades. Heat stress severely affects the milk production globally. The occurrence of sporadic weather event and extreme hot and summer days resulted in huge economic loss in livestock industry (Frumhoff *et al.* 2007). Even for the farms with proper management systems, these sudden changes in the climate results in rapid reduction in productivity resulting in lower profitability. Therefore, it is essential to adopt appropriate management and mitigation measures to counteract these effects.

As per IPCC, 1.8°C rise in mean global temperature results in a 1.39 percent decline in livestock production particularly in Southeast Asia and further the predicted increase in temperature may exacerbate the condition (Darwin, 2001). It has been projected that the global milk production should be increased by 2 percent to meet the demands of livestock products (Darwin, 2001). A study on impact of heat stress on the economy of US livestock industry conducted by Key and Sneeringer (2014) observed that the total annual cost for livestock industry would be between $1.69 and $2.36 billion and about 40-60 percent cost aroused from dairy sector alone. The US dairy industry anticipates a substantial milk production loss of

6.3 percent by the end of 2080 (Mauger *et al.*, 2015). The variables that were considered for the calculation of economic loss were dry matter intake, milk production, reproduction, number of culled animals, and death of cows.

2.5 Impact of heat stress on Reproduction in livestock

Reproductive efficiency of livestock is of foremost economic concern in the development of agricultural sector. The integrated role of several hormones such as gonadotrophin-releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) establishes the normal functioning of reproductive system in animals. In order to maintain homeostasis and normal metabolic functions, every heat stressed animal has a tendency to compromise the productive and reproductive functions so as to deviate the energy for vital body functions. However in the case of tropical and subtropical regions, the upper limit of ambient environmental temperature exceeds during the summer months. Therefore, an elevated ambient temperature interrupts the normal physiological and reproductive performances (Torres-Júnior *et al.*, 2008). The disturbed reproductive functions are not only attributed to the reduction in feed intake and related metabolic responses but also due to the direct effect of thermal stress on reproductive endocrine mechanisms (Ronchi *et al.*, 2001). There are cascade of events that determines the reproductive functionality of HPG axis during heat stress in livestock. Heat stress influences the secretion of GnRH and inhibits the ovarian activities.

Heat stress is a major threat to all the stages of reproductive performance beginning from the pubertal development to conception and embryo development (Williams and Walsh, 2010). Both ranges of temperature extremes (low and high) delay the onset of puberty in both males and females. Heat stress also has a substantial influence on endocrine responses that may result in increased rate of decreased follicle and oocyte maturation, fetal abortion, shortened gestation period and reduced birth weights during the postpartum reproductive cycle (Nardone *et al.*,

2010). According to the studies of Wolfenson *et al.* (2000), heat stress caused negative effects on secretory activities of endometrium. Higher temperature affected the production of gonadotropins that lead to a considerable decrease in the secretion of estrogen and progesterone (Scholtz *et al.*, 2013). In buffaloes, a temperature rise of 2°C leads to desynchronization of endocrinal activities of pinealhypothalamo-hypophyseal-gonadal axis altering the functions of hormones (Kebede, 2016).

2.5.1 Estradiol synthesis

Estradiol, the primary reproductive hormone is responsible for the estrus expression in animals. Heat stress hampers the estradiol synthesis due to the significant reduction in steroidogenic capacity of granulosa and theca cells of follicles (Bridges *et al.*, 2005). Hence, the reduced production of estradiol as a result of overheating of body lowers the expression of estrus (Masoumi and Derensis, 2013). In addition, heat stress exposure of animals during the pre-partum period reduced the level of thyroid hormone and placental estrogen. The changes associated with follicular dynamics (Wolfenson *et al.*, 1995) and estradiol production consequently lead to reduced length of estrus (Hansen and Areechiga, 1999). The decline in circulating concentration of estrogen affected the intensity and behavioral signs of estrus (Dash *et al.,* 2015).

2.5.2 Follicle Stimulating Hormone (FSH)

Heat stress has a direct impact on ovary. Heat stress decreases the sensitivity of ovary towards the stimulations by gonadotrophins (Sartori *et al.*, 2009). Continuous exposure of animals to 12 h of heat stress reduced the androstenedione secretion from thecal tissues of preovulatory follicles (Biran *et al.*, 2015).

The exposure of animals to the summer heat stress during the pre-ovulatory period resulted in hindered secretion of inhibin and increase in the concentration of FSH (Roth *et al.*, 2000). The elevated FSH level indicates the decline in inhibition of

negative feedback from immature follicles which affects the reproductive efficiency (Khodaei *et al.,* 2011). Therefore, the upsurge of plasma FSH concentration altered the mechanism of follicular dominance (Wolfenson *et al.*, 1995). However, Ronchi *et al.* (2001) did not found any significant changes in the blood level FSH concentration during summer.

2.5.3 Luteinizing Hormone (LH)

According to Williams and Walsh (2010), the concentration of luteinizing hormone (LH) in the peripheral blood is variable when the animal is exposed to heat stress. The LH synthesis reduces as the severity of environmental temperature increases in a thermal stressed cow. Glucose or feed availability acts as a crucial factor that determines the pulsatile secretion of LH (Bucholtz *et al.*, 1996). Therefore, an alteration in the feed availability and feed intake due to constant exposure of dairy cows to heat stress contributes to the lower fertility by preventing the normal ovulation process (Ahmed *et al.*, 2015). Warmer environmental conditions suppressed the pulsatile secretion of LH and also a pre-ovulatory surge of LH (Kadokawa, 2007). These consequences are related to the direct impact of heat stress on HPG axis or indirect effect on feed intake and concentration of several hormones that governs the reproductive performance. This immediate effect on LH release increases the variability of interval between estrus and ovulation. Hence, chances of untimely inseminations are high during summer due to the irregular estrus manifestation. Further, unprecedented changes in the LH release leads to a greater risk of ovulatory failures. High ambient temperature can also be a reason for the development of ovarian cyst (Stradaioli *et al.*, 1994).

2.5.4 Progesterone secretion

In heat stressed cattle, plasma progesterone level reduced during the luteal phase and thus it has a subsequent effect on estrous cyclicity by compromising the follicular development which further lead to abnormal oocyte maturation and early

embryo death (Khodaei, 2013). The serum progesterone concentration decreased to 1ng/ml at THI value 74 and it was found that further increase in temperature augmented the further progesterone level drop (Schüller *et al.*, 2017). Therefore, low circulating level of progesterone lead to early manifestation of estrus resulted in difficulties in implantation (Khodaei *et al.*, 2011).

2.5.5 Prolactin secretion

Prolactin, a thermo-sensitive reproductive as well as metabolic hormone increased significantly during the animal's exposure to summer heat stress (Lupoli *et al.*, 2001). Prolactin has an essential role in maintaining the homeostasis through the dissipation of excess heat load (Kauffman and Hughson, 1988). However, the associated mechanism has not been properly understood. Alterations in the levels of prolactin deteriorated the follicular development and the competence of oocytes (Lebedeva *et al.*, 2014).

2.5.6 Melatonin secretion

Melatonin is the major hormone that plays an essential role in photoperiodic regulation of reproduction which is seasonal phenomena in animals (Gwazdauskas *et al.*, 1981). During summer induced heat stress, the melatonin secretion decreases as a result of long day pattern of photoperiod. The reduced melatonin has a direct influence on the functions of granulosa cells and summer subfertility which can be altered as a result of heat stress (Wang *et al.*, 2012).

2.5.7 Follicular development

Heat stress imparts significant changes in the follicular dynamics of the normal reproductive cycle (Wolfenson *et al.*, 2000). Wilson *et al.* (1998) reported that the heat stress has a severe influence on the follicular development as a result of reduced synthesis of steroid hormones. Heat stress influenced follicle selection and

length of follicular waves (Roth *et al.*, 2001). These variations further deteriorate the oocyte growth. Seasonal heat stress in farm animals was reflected on the reduced size of dominant follicles while increasing the number and size of medium sized subordinate follicles (Paes *et al.*, 2016). This unusual growth of subordinate follicles controls and dominates the growth of mature follicles preventing them from complete dominance (Bajagai, 2011), which ultimately lead to the ovulation of aged follicle carrying oocytes with less competency (Mihm *et al.*, 1999). However, the researchers are uncertain about the impact of heat stress on smaller follicles. Though, there are reports suggesting an increase in the number of small immature follicles (Trout *et al.*, 1988), there are also reports pertaining to their reduction in the ovary (Wolfenson *et al.*, 2000; Hansen, 2011). Therefore, the dominance of weaker follicles are associated with an early emergence of second wave dominant follicles.

In heat stressed cows, the high temperature disrupts both the nuclear and cellular processes involved in oocyte maturation which in turn compromises fertilization, embryo maturation and development (Gendelman and Roth, 2012a). Thermal stress results in the stimulation of apoptosis, restricting the nuclear maturation and cytoskeleton of oocyte (Roth and Hansen, 2005). Similar reports of declined oocyte quality and decreased fertility was established by Lacerda and Loureiro (2015). Additionally, the follicular diameter decreased by 0.1mm with each point increase in THI (Schüller *et al.*, 2017). The escalated temperature has also a direct impact on oocytes (Ferreira *et al.*, 2011). Furthermore, the concentration of metabolic hormones and growth factors (which are essential for follicular development) decreased in the blood in response to heat stress and its associated effect on dry matter intake (DMI) (Igono *et al.*, 1988). De Rensis *et al.* (2002) in a study on dairy cattle concluded that insulin, insulin like growth factor-1 (IGF-I), glucose concentrations declined in the heat stressed cattle during the postpartum phase. As insulin plays a major role in normal follicular development as well as determination of oocyte quality, the rapid decline in insulin level affected the fertility

of animal. Similarly, IGF-1 and glucose stimulates the process of implantation and also acts as a metabolic fuel for ovary. Therefore, deviations of these crucial components from the normal level affect the reproductive functionality of livestock.

2.5.8 Heat stress impact on estrus

Heat stress has a prominent impact on length and intensity of estrus (Ahmed *et al.*, 2015). Reduced motor activity and estrus manifestation are the clear evidences of heat stress during peak summer season which induces anestrus and silent ovulation (Hansen and Arechiga, 1999). Rutledge (2001) reported that 80 percent of the estrus goes unnoticeable during summer in cattle. The number of anovulatory follicles increases with cystic ovaries and also probabilities of double ovulations and twin birth were observed during heat stress (López-Gatius and García-Ispierto, 2010).

Several studies established a significant impact on the mounting behavior of animals during estrus. Higher body temperature considerably reduced the behavior of mounting during estrus (Gwazdauskas *et al.*, 1983; Pennington *et al.*, 1985; Schüller *et al.*, 2017). The color and presence of vaginal discharge is a good indicator for an animal in estrus (Hassig *et al.*, 2006). The estradiol is produced from the follicles with larger diameter (≥12) on the day of estrus, responsible for the secretion of cervicovaginal mucus which resulted in the higher amount of estrus discharge (Senger, 2003). Heat stress possibly reduced the amount of estrus discharge and this decline was further assisted by the increase in THI on the day of estrus (Schüller *et al.*, 2017). Summer season induced heat stress resulted in the reduction of estradiol during the first day of estrus and lesser manifestation of heat in Indian buffaloes (Upadhyay *et al.*, 2009).

2.5.9 Conception rate

Gwazdauskas (1985) established that heat stress has a harmful effect on livestock by reducing the conception rate by 20 percent to 27 percent. Paula-Lopes *et al.* (2012) reported that the conception rate decreased by 6.9 percent when the uterine temperature rised by 0.9°C. In temperate climate, the conception rate dropped when the THI value crosses 56 in dairy cows (Schüller *et al.*, 2014). Similarly, exposure of cows to high environmental temperature the day after insemination reduced the conception rate (Nabenishi *et al.*, 2011). During summer, a 90-day non-return rate to the first service was recorded in lactating dairy cows and simultaneously a drop in conception rate (20-27 percent) was also established (Al-Katanani *et al.,* 1999; Chebel *et al.*, 2004). Severe heat stress culminates in reducing the rate of successful inseminations by 10-20 percent (Roth *et al.*, 2000). Further when the influence of heat stress subsides, it takes around 40-60 days for an animal to return to its normal functioning of reproductive cyclicity. Additionally, during severely hot summer months, service period increased with increase in THI (THI>75) in Murrah buffaloes (Dash, 2013). In buffaloes, the conception rate has a significant reduction when they are subjected to a THI above 75 (Kumar and Gandhi, 2011). The fertility rate decreases in high yielding dairy cows due to the severe impact of rapidly rising ambient temperature. The fertility of lactating cows were usually found to be more compromised due to thermal stress. The causes for this fertility associated drawbacks could be attributed to the high level internal metabolic heat production during summer season (Takahashi, 2012; M'hamdi *et al.*, 2012).

The GnRH administration during early estrous helped in inducing LH surge, which successfully improved the conception rate (Ullah *et al.*, 1996). But according to the findings of Schmitt *et al.* (1996) supplementation of progesterone after insemination did not induced any difference in the heat stress associated adverse impact on the conception rate. However, the administration of progesterone to non-heat stressed cattle aided the conceptus development. Therefore, it can be

emphasized that conceptus damage occurred when the animal is under high heat stress during the period between the day of estrus and up to 7th day of pregnancy (Ealy *et al.*, 1993).

2.5.10 Pregnancy rate

In subtropical climate, increase in mean THI (above 69) reduced the pregnancy rate by 34.1 percent to 15.7 (El-Wishy, 2013). Similarly, Khan *et al.* (2013) established that pregnancy rate considerably decreased from 32.6 percent to 20.5 percent when animals are maintained above their TNZ. The pregnancy rate substantially decreased when the animal is exposed to THI threshold between 51 to73. The lower conception rate was recorded at a THI value of 72 during the day of estrus (Schuller, 2017). Further, heat stress also reduced the production of interferon- tau which adversely affected the signaling of pregnancy recognition (Bilby *et al.*, 2008). In addition, Bilby *et al.* (2008) found an upsurge of concentration of endometrial PGF2α during heat stress that can threaten the maintenance of pregnancy.

Cattle that are subjected to heat stress during their late gestation period yields calves with lower birth weight (Collier *et al.*, 1980). This is related to a reduction in circulating concentration of thyroxine, prolactin, growth hormone and glucocorticoids which ultimately affects the feed intake and metabolic rate (Avendano-Reyes *et al.*, 2006). Additionally, increased concentration of nonesterified fatty acid (NEFA) during heat stress altered the growth of udder and placenta, nutrients transferred to the fetus and milk production (Collier *et al.*, 1980).

2.5.11 Embryo development

Embryo loss is another outcome of the adverse effects of heat stress on reproductive efficiency of livestock. The bovine embryos are sensitive to maternal heat stress during the first two weeks after breeding (Ryan *et al.*, 1993). Exposure of

farm animals to high temperature during the first 3 to 7 days of pregnancy results in reduced embryonic viability and development (Hansen, 2013). Elevated body temperature restricts the development of zygotes and embryos resulting in embryo loss. Heat stress influences the protein synthesis and transcript formation for the embryonic development. The changes in intrauterine temperature as a result of heat stress compromised the blood flow towards the uterus (Roman-Ponce *et al.*, 1978). Even though the studies established the reduction in uterine blood flow during heat stress, the researchers could not elucidate the reason behind this so far (Schüller *et al.*, 2017). These changes affected the embryos at their pre-attachment stage (Ray *et al.*, 1993). However, this magnitude of the impact reduced as the embryo develops (Ealy *et al.*, 1993).

2.6 Importance of studying the heat stress impact on indigenous livestock breeds

The contributions of indigenous livestock breeds are inevitable for the livelihood of economically backward communities in the developing countries. The production level of any genotype during the extreme environmental condition depends on an integrated role played by different traits in an animal (Nyamushamba *et al.*, 2017). Indigenous livestock breeds are well known for their adaptive capability to the agro-climatic conditions from where they have evolved. The indigenous livestock breeds of tropical and sub-tropical countries exhibit a better rate of survivability and productive traits including growth, milk production, and reproduction (Silanikove, 2000).

Indigenous goat breeds possess superior adaptive traits to survive in their native agro-ecological zones. However, there are certain breeds which could adapt and produce better in different agro-ecological zones (Helal *et al.*, 2010; Archana *et al.*, 2018; Pragna *et al.*, 2018). The indigenous breeds are superior over the exotic breeds which are native to temperate region. Further, the indigenous breeds are capable of withstanding a combination of harsh environmental conditions including

high temperature, drought, floods and spread of epidemic diseases and parasites (Kohler-Rollerfson, 2004). In addition, they are also capable of thriving well in extreme environmental conditions and are able to feed on limited pasture where the high yielding animals fails to survive in such conditions (Akinyi, 2008). Kadim *et al.* (2007) reported that indigenous sheep breeds of tropical and sub-tropical regions are able to breed throughout the year even though their sexual activity is restricted due to their exposure to heat stress during summer season. There are very less reports on the impact of heat stress on the physiological indices, carcass traits and meat quality in indigenous sheep breeds of tropical and sub-tropical regions. The physiological adaptabilities such as superior digestive capability, efficient utilization of nitrogen and efficient water usage in goats of tropical regions have been elucidated so far, which indicates their superior trait of withstanding testing environmental condition (Silanikove, 2000).

Generally, the indigenous breeds perform better with respect to calving rates and survivability than the exotic breeds. Exotic breeds generally produce heavier calves than the indigenous breeds but they suffer in terms of maintaining the growth rate overcoming the environmental conditions (Mpofu, 2002). The production potential of indigenous breeds are less compared to the exotic high producing breeds. But they ensure a constant production throughout the testing conditions where the exotic breeds fails to provide sufficient output. Most of the high yielding exotic cattle breeds were originated from the temperate regions and this could be the reason for their low productive capabilities when they are exposed to hot and humid environment. Indigenous cattle posses diversified genetic pools that are useful to assure a better sustainable production by surpassing all the future challenges related to climate change (Hanotte *et al.*, 2010). Elevated temperature and changes in soil condition affects the quantity and quality of fodder available to livestock. In country like Africa, the indigenous cattle breeds are highly tolerant to such changes and they are genetically equipped with better feed utilization characteristics which prepares

them to be heat and drought tolerant (Shabtay, 2015). For instance, Nguni cattle breed (small to medium sized) of South Africa requires less nutrients for the maintenance of body functions, they are capable of walking long distances in search of feed and water (Strydom *et al.*, 2001), optimal utilization of the nutrients from the available natural resources and good browsing abilities helps them to survive in harsh environments in comparison with European cattle breeds (Marufu *et al.,* 2011). The amount of nutrient required for the proper physiological functions of the body depends on the body size of the animal (Ndlovu *et al.*, 2009). The smaller body size of indigenous breeds relates to the influence of evolutionary mechanism and natural selection that synchronizes the genetic potential of the animal to the available feed resources (Nyamushamba *et al.*, 2017). Nguni cattle of Southern Africa (Muchenje *et al.*, 2008), Kenana cows indigenous to Sudan (Bahbahani *et al.*, 2018), Sahiwal cattle of India (Bhanuprakash *et al.*, 2016), Mashona and Tuli of Zimbabwe (Khombe, 2002) are some of the indigenous well adapted livestock breeds to harsh extreme climatic conditions.

Even though indigenous livestock breeds possess a genetic merit of coping with extreme environment, these ability was not validated in certain breeds (Sansthan and Köhler-Rollefson, 2005). Currently, the genetic erosion of indigenous livestock breed is a major threat to the food security throughout the globe (Assan, 2013). Therefore, adequate measures have to be adopted and implemented to conserve such pure breeds (Assan, 2013). Hence, it is essential to preserve the gene pool of indigenous breeds in order to sustain livestock production in this changing climate scenario.

2.7 Goat as ideal animal model from climate change perspective

Livestock sector contributes 15 percent of revenue to the total family income of poor and marginal farmers in tropical regions (Chokerah and Horvath, 2012). Small ruminant rearing is relatively easy and less expensive as compared to other

livestock species. They provide huge turnover with low initial investment which is the major reason for the promotion of small ruminant production in developing countries (Pollot and Wilson, 2009). Small ruminants constitute 56 percent of the total global ruminant domestic population (FAOSTAT, 2013). On a global average, 56 percent of the total small ruminants are located in arid zones, 27 percent in temperate zone and 21 percent is located in humid regions (Otte and Chilonda, 2002).

Out of the whole livestock population, small ruminants especially goats has a substantial role in improving the income and nutrition of farmers throughout the world (Mlambo and Mapiye, 2015). Globally, large scales of goat milk are produced from developing countries (FAO, 2013). Particularly in tropical country like India, goats are considered the ideal animals due to their higher thermo-tolerance, drought tolerance, highly disease resistance and their ability to survive in low pastures. Moreover, their smaller body size is highly appreciable, which helps the farmers to integrate them to any farming systems (Amankwah *et al.*, 2012). Additionally, their reduced body size ensures the farmers lower economic loss and guarantees a better productive and reproductive capability of animals with low initial investment in contrast to the large ruminants (Aphunu, 2011). Further, Silanikove (2000) opined goats as the ideal climate animals because of their ability to tolerate high ambient temperature than other livestock species. The indigenous breeds that are located in the tropical and arid regions of the world are well known for their ability to adapt to hot climate in comparison with those reared in temperate regions (Marai *et al.*, 2007). Moreover, goats can withstand higher THI values than other livestock breeds due to their superior ability to conserve body water and reduced body size (Silanikove, 2000). It has been established that the goat breeds that are native to tropical as well as desert environment are highly efficient in coping with the climatic change by adopting certain adaptive mechanisms (Silanikove, 2000). Studies established the deleterious effect of heat stress on large ruminants but studies emphasizing the extreme influence of heat stress are relatively less in goats. Further, goats are well

known for their improved feed conversion efficiency in which low quality feed is metabolized to high quality protein (Silanikove and Koluman, 2015). Their typical browsing nature (they can feed on any type of pastures) helps them to survive in any kind of agro-ecological zone (Shilja *et al.*, 2016). Therefore, the aforementioned statements recapitulates that goat is an ideal climate animal that can adapt to various harsh climatic conditions and several extreme environments with least production loss in comparison with large ruminants (Silanikove and Koluman, 2015).

2.8 Malabari goat

Malabari goats also known as Tellicherry goats are the indigenous goat breeds of Kerala, India. They are widely located in the districts of Calicut, Wayanad and Malappuram along the coastal lines and midlands. These breeds are also reared in different parts of Tamil Nadu. Kaura (1952) reported that the Malabari breed is the goat breed developed from the mixed population several Arab Indian goats such as Cutch cross and Tellicherry. They are small to medium sized animals having coat color ranging from white to black and also combinations of white and brown or black and brown. They are the goat breeds reared primarily for meat purpose. The average body weight of these breeds ranges from 8.83 kg (during 3 month age) to 36.31kg (18 months and above). Among the Malabari breeds, 40 percent of the goats has long hair and 20 percent of both males and females have beard. The typical physical characteristics of this particular breed includes pinkish red coloured muzzle, pinkish white eye lids, straight nose and slightly twisted and backward directed horns sometimes they are curved to touch the skin, the ears are folded and directed downwards. In some animals, the tips of ears are curved or folded, small and thin tail and their udder is small with medium sized teats. Malabari goats have originated from hot humid tropical zone. They are capable of withstanding harsh environmental conditions. These goat breeds are prolific breeders and they possess unique genotypic characteristic of giving birth to multiple progenies and providing higher milk yield (Verma *et al.*, 2009). These breeds attain puberty in five to six months of

age and they are able to provide 1 to 2 litres of milk during that period of time. Verma *et al.* (2007) reported a possibility of 50 percent twinning, 25 percent triplets and 5 percent quadruples in Malabari goat. The average kidding intervals in these breeds are 9.47±0.11 months (Raghavan *et al.*, 2004) however, the recorded kidding interval was found to be 315 days (Raghavan *et al.*, 2017). Malabari breed of goats plays a major role in providing financial assistance to the poor farmers of Wayanad district. The farmers claim that breeding of Malabari goat is highly profitable because of its low management cost in comparison with large ruminants.

2.9 Factors affecting the expression pattern of different reproduction related genes with special reference to climate change or heat stress in livestock

The energy exchange process between animal and environment is altered by the interference of climatic variables, animal factors and thermoregulatory mechanisms (Maurya *et al.*, 2009) which has a significant effect on expression pattern of respective genes. Reproduction is an energy demanding process which directly depends on the action and response of genetic mechanisms, management practices and environmental changes (Aguiar *et al.*, 2013). The optimal functioning of reproductive mechanism primarily depends on several factors including both biotic and abiotic variables. Major factors influencing the gene expression pattern are climatic stressor, nutritional status and age of the animal and disease.

Climatic factors which can influence reproductive gene expression includes AT, RH and wind speed. These are the major external variables that influence the homeothermy (which is very essential to maintain the reproductive functions). The reduction in the reproductive efficiency and production capabilities are directly associated with the effects of heat stress (Key and Sneeringer, 2014). Elevated temperature reduced the follicular development and endometrial secretions. The impact of heat stress and the heat tolerant mechanism on fertility is considered to be influenced genetically. The environmental stresses markedly compromise the

genetic potential of an animal resulting in affecting their capability to reproduce (Ross *et al.*, 2017).

Apart from climatic variables, nutrition is also a crucial factor influencing livestock reproduction. Nutrition also has an important role in cellular and endocrine regulation of reproductive performance (Sejian *et al.*, 2011). Improper and inadequate nutrition in livestock leads to nutritional stress. Combined effect of both heat and nutritional stress will negatively affect the fertility of the animal. The impact and magnitude of nutritional stress varies from species to species and season to season (Sejian *et al.*, 2011). Particularly in young animals, the restricted feed availability severely affects the development of gonads and neural tissues (Martinez *et al.*, 2012). In addition, unavailability of adequate nutrition during postpartum period affects the estrus expression and rebreeding performances in cattle (Alam *et al.*, 2010).

The genetic make-up of an animal substantially contributes to the productivity as well as reproductive abilities in coordination with the influence of other external factors (Walthall *et al.*, 2016). In addition, breed difference plays a major role in expressing the desired productive and reproductive traits (Penasa *et al.*, 2014).

Age of an animal also has a significant role in the reproductive functions due to the gradual changes in metabolic and cellular levels. It has been established that the animal will be at high risk of culling when it is calved during older age (Páchová *et al.*, 2005). Further, it has been established that both cow and bull should be of appropriate age for insemination so that the conception rate would be appropriate. This shows that age is an important factor to influence the reproductive success in farm animals (Zavadilová and Stipková, 2013).

2.10 Expression pattern of different reproduction related genes in goats/ other livestock

Even though there are several reports pertaining to the alterations in the concentration of steroids and changes in the follicular dynamics, studies regarding the cellular level expression pattern of reproduction related genes subjected to heat stress are very scarce. The following discussion emphasis on the cellular level changes in reproduction of livestock as a result of heat stress.

A significant increase in the expression pattern of genes encoding FSH and FSHR was reported in ewes supplemented with lipopolysaccharides (Herman *et al.*, 2010). Additionally, FSHR expression was reported to be higher in prolific goats indicating the significance of this receptor in establishing the reproductive efficiency in ewes (Cui *et al.*, 2009). However, Ozawa *et al.* (2005) reported that the constant exposure of goats to heat stress restricted FSH stimulations to enhance the aromatase activity of follicles which lead to a decline in the expression of FSHR expression which in turn decreased the circulating level of estradiol concentration.

In contrast to FSH and FSHR, Herman *et al.* (2010) reported significant reduction in the expression pattern of genes encoding the LH and LHR in ewes. Further, Ozawa *et al.* (2005) established a significant reduction in the expression of LH receptor (LHR) in heat stressed follicles in goat (Ozawa *et al.*, 2005). However, no substantial evidences were found in the bovine thecal cells for the expression pattern of LHR (Roth, 2008). The reduction in the expression pattern of LHR could be attributed to the reduction in the steroidogenic activity and disrupted follicular dynamics (Das *et al.*, 2016).

Similar to FSH and FSHR Herman *et al.* (2010) reported increase in the expression pattern of prolactin and prolactin receptors in ewes (Herman *et al.*, 2010). From a study conducted on the two breeds of cow Jersey and Nellore,

cyclooxygenase-2 (COX2) gene expressed in the trophectoderm of preimplantation embryos was tend to decrease as a result of constant exposure to the heat stress. The decline in the expression pattern of COX2 was observed in the embryos of Jersey cow but no significant change was observed in Nellore embryos (El-Sayed *et al.*, 2006).

Nteeba *et al.* (2015) established higher expression pattern of insulin receptor gene expression in the ovaries of gilts during heat stress. Further, these authors also established a significant impact of heat stress on the expression pattern of genes encoding several steroidogenic enzymes in the ovary.

2.11 Biological markers for assessing the impact of heat stress on animal reproduction

In a stress assessment study, measurement of a single indicator is not always reliable. Therefore, assessment of multiple indicators or biological markers helps in the better understanding of the response of animal welfare to different stresses. The use of molecular (biological) markers plays an essential role in the improvement of production traits in livestock (Mirkena *et al.*, 2010). Generally, changes in the hormonal profile which is often accompanied with the alterations in behavioral or physiological changes can be considered as a popular measure of stress (Kumar *et al.*, 2012). There are plenty of traditional approaches to assess the animal response to heat stress and for the betterment of their traits. However, these techniques are inadequate to decipher the role of several genes that are linked with the genetic pathways and associated stress responses (Kumar *et al.*, 2012). Therefore, a sound knowledge about the basic biological processes, changes in physiological responses, expression of specific genes, protein and markers involved in the adaptation process paves the way for the researchers in developing novel techniques for improving the livestock production and reproduction.

Biological marker is the term which refers to a variation of specific DNA between different individuals that is often associated with certain characteristics (Singh *et al.*, 2014). The markers have a biological property in which they can be identified or detected in certain part of the body likely, the blood or tissue of specific organs during the treatment (Deb *et al.*, 2012). It also indicates the change in expression or state of a protein which is highly related to the susceptibility of the animal to any stressed condition. Identification of appropriate markers controlling a specific phenotypic or genotypic trait in animals is the major challenge faced in the field of molecular biology (Singh *et al.*, 2014).

Selection of biological markers for desired production traits at the molecular level helps in reducing the cost of selection of programs. Consequently, the researchers are focusing on gene mapping and identifying the genes involved in the endocrine regulation of reproductive performance and exploring their expression and polymorphic patterns of these markers (Deb *et al.*, 2012). Considering the reproductive potential of livestock population, FSHR (Yang *et al.*, 2010), LHR (Yang *et al.*, 2012), inhibin (Tang *et al.*, 2011), progesterone receptor (Yang *et al.*, 2011), ESTR, are identified as the major genes that has an influential role in the ova production and even they were identified to be the potential markers for the super ovulation trait. Therefore, any kind of stresses particularly heat stress was able to alter the expression patterns of the genes or concentration of the respective hormones. As discussed in the above sections, heat stress is the major challenge intimidating the reproductive performances that can influence the endocrine as well as cellular level responses in livestock. Hence, GnRH (Siregar *et al.*, 2017), FSH (Sheikh *et al.*, 2017), LH (Bun *et al.*, 2018), estradiol (Ghosh *et al.*, 2017), progesterone (Allen *et al.*, 2015), prostaglandins (Luo *et al.*, 2016) were found to be the ideal indicators to reflect the compromised reproductive efficiency in heat stressed livestock.

Sustaining livestock production in the changing climate scenario is the need of hour which can help to deal a lot with the growing global food security issues. The above literature review clearly indicates the dearth of information on the molecular level control on reproductive activities in farm animals. Research efforts are therefore needed to bridge this gap in information to help to provide a better understanding on the molecular mechanisms controlling reproductive activities during heat stress exposure in animals. This can help the policy makers to develop better amelioration strategies to sustain the reproductive efficiency in the changing climate scenario. Goats being hardy animals and with the low initial investments required they are considered to be the futuristic animals to withstand climatic impact. Therefore, research efforts pertaining to understanding the hidden intricacies of heat stress associated reproduction related gene expression patterns can help to identify suitable biomarkers which can be used in breeding programs to evolve a breed which can reproduce optimally in the changing climatic condition.

These are all the efforts which help to prevent the glooming food security issues in the face of growing global human population.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental animal

Twelve one year old Malabari female goat breeds were used for the study. It is an indigenous goat breed of Kerala, which is well known for their adaptive nature in hot and humid conditions of Kerala. It is dual purpose breed predominantly reared for meat purpose. Plate 3.1 describes the breed characteristics of Malabari goat breed.

3.2 Experimental design

The study was conducted in the experimental livestock unit of the ICAR-National Institute of Animal Nutrition and Physiology (ICAR-NIANP), Bengaluru, India. Twelve healthy female Malabari goats were used in the study and the animals were allocated into two groups of six animals each, MC (n=6; Malabari control), and MHS (n=6; Malabari heat stress). The duration of the study was for a period of 45 days. The animals were provided with the diet comprising of 60% roughage (Hybrid Napier) and 40% concentrate. The concentrate mixture comprises of Maize, wheat bran, soybean meal, mineral mixture and common salt to the tune of 36 Kg, 37, Kg, 25 Kg, 1.5 Kg and 0.5 Kg respectively for a 100 Kg concentrate mixture. During the experimental period, the MC animals were kept in the shed in comfortable condition and the MHS animals were kept outside the shed exposed to summer heat stress between 10:00 h to 16:00 h. The temperature-humidity index (THI) was calculated by the formula as described by McDowell (1972). The MC animals were fed and watered inside the shed while MHS animals were provided with feed and water when they were kept exposed to heat stress in outside environment. The cardinal weather variables were recorded both inside and outside the shed twice daily. At the end of the study, the animals were slaughtered and their uterine samples were

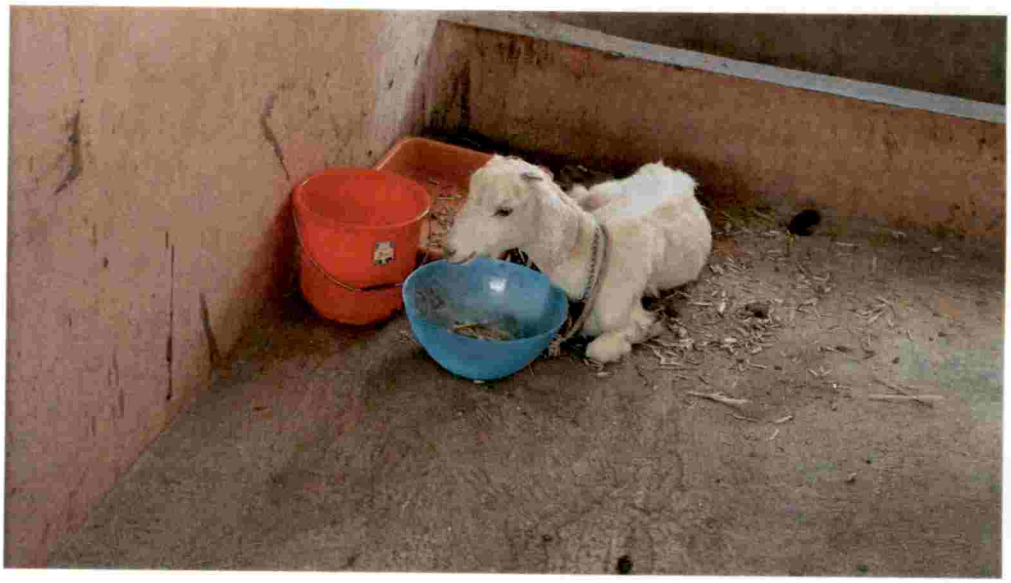
collected in aseptic condition for subjecting them to gene expression. The ownership of the experimental animals was with the Director of ICAR-NIANP. These animals were maintained in the experimental livestock unit of the institute and they were used in the study after taking permission from the institute director. All handling and management procedures performed in the study were in accordance with the ethical standards of the Institutional Animal Care and Use Committee (IACUC, ICAR-NIANP). In addition, the study was conducted after obtaining approval from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India for subjecting the animal to heat stress (NIANP/IAEC/2/2017).

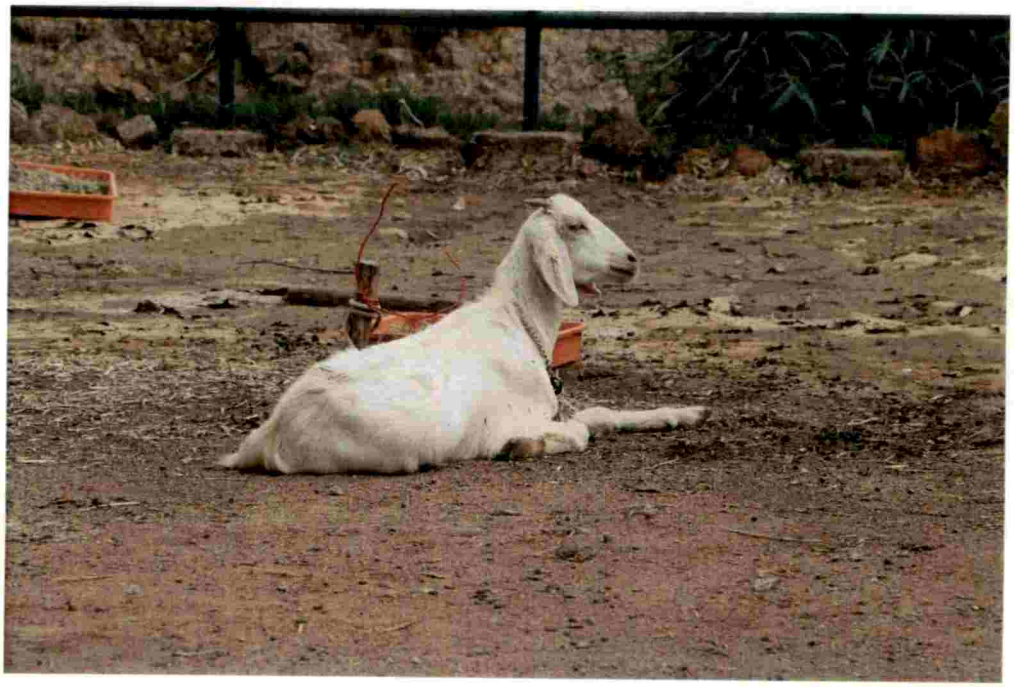
3.3 Expression of reproduction related genes in uterus

The animals were slaughtered at the end of the study and representative uterine samples were collected from each animal. Immediately after collection the tissue samples were cut into small pieces and washed in phosphate buffered saline. The samples were then kept immersed in RNA shield (Zymo Research, USA) and snap chilled in liquid nitrogen (LN_2) and kept stored at –80 °C till further use.

The tissues samples were thawed after removing them from RNA shield (Zymo Research, USA) and processed for RNA isolation. The total RNA was isolated from tissues using the GeneJET RNA Purification Kit (Thermo Scientific, Lithuania) and the procedure was carried out as per manufacturer's protocol. Total RNA was treated with DNase (TURBO DNA-free, Ambion, USA) to eliminate the genomic DNA contamination. The purified total RNA samples were stored at –80 °C until cDNA synthesis. The Maxima first strand cDNA synthesis kit (Thermo Scientific, Lithuania) was used to reverse transcribe the total TNA into cDNA. The

Plate 3.1 Pictorial representation of Malabari goat breed both inside and outside the shed





cDNA was subjected to real time quantitative polymerase chain reaction (RTqPCR).

Specific primers were synthesized for the target genes using NCBI primer design software (Primer3, http://bioinfo.ut.ee/primer3/) and Primer3 and BLAST websites (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) were used to check the specificity of the primers. Different primers used for amplifying the target regions of various genes in the study are described in table 3.1. The relative quantitative expression patterns of target genes were studied using SYBR green chemistry (Maxima SYBR green qPCR master mix, Fermentas, USA) using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene as internal control. The relative expression patterns of target genes in comparison to the housekeeping gene were analyzed as per the formula 2ΔΔCT (Shilja *et al.,* 2017).

3.4 Histopathological observation

All the animals were slaughtered at the end of the study period and their uterine tissues were collected for histopathological sectioning. Care was taken to collect representative tissue sample from the same site of uterus in all experimental animals. Immediately after collection the tissue samples were kept in 10% formalin and processed for obtaining histopathological sections. The tissue sections were stained using Haematoxylin and Eosin (H and E) stain as per the method described by Luna (1986). The results were interpreted by comparing between MC and MHS sections and the representative lesions were photographed. Based on the degree of histological changes the scores were given. The scoring pattern for the histological section was based on the method of Gibson-Corley *et al.* (2013). Three different scoring patterns were followed on a scale of 0-3 point as 0-normal; 1- mild; 2moderate and 3-severe changes.

3.5 Statistical analysis

The quantitative relative expression pattern between target genes and housekeeping gene were analyzed by one-way analysis of variance (ANOVA) using SPSS version 18.0 software. The level of statistical significance was set at $P<0.05$. Pearson's correlation coefficient test was used to assess the correlation coefficient between the THI and all genotypic traits using R^2 values by setting two levels of statistical significance at $P<0.01$ and $P<0.05$. Again one-way ANOVA was used to assess the degree of changes associated with histological sections between MC and MHS groups.

Table 3.1 PCR primer pairs and reaction condition used in gene expression study

Gene ID	Primer	Primer sequence (5"- 3")	TA (°C)	Accession No
FSHR	F	ATGCGGTCGAACTGAGGTTT	60	NM_001285636.1
	R	GGGCAGGTTGGAGAACACAT		
LHR	F	TTCCACTAAACTGCAGGCCC	60	NM_001314279.1
	R	CAGTGGCTGGGGTAAGTCAG		
ESTR	F	ATACGAAAAGACCGCCGAGG	60	GQ 358923.1
	R	GGTTGGCAGCTCTCATGTCT		
COX2	F	GCCTAGCACTTTCGGTGGAG	56	JN 743538.1
	R	CCGTTTTGGTGAGGTGCGTA		
PGF2α	F	AGTTTGGAACAGATGCCCCC	60	XM 005678194.3
	R	GCTGGCCACTCAAGTCATCT		
PGE2	F	TGCTCCTTGCCTTTCACGAT	60	NM_001314255.1
	R	AGGAGGTCTCAGGATGGCAA		
GAPDH	F	GGTGATGCTGGTGCTGAGTA	60	AF030943
	R	TCATAAGTCCCTCCACGATG		

Plate 3.2 Pictorial representation of laboratory activities of gene expression study

RESULTS

CHAPTER 4

RESULTS

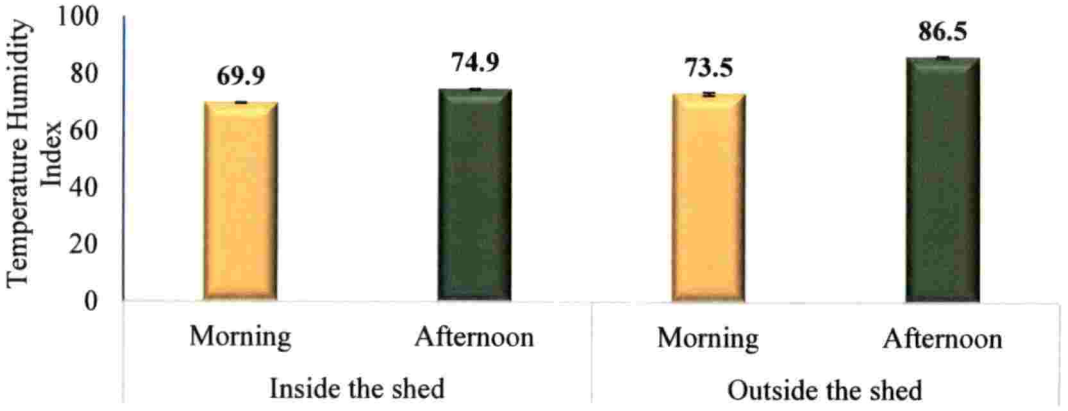
4.1. THI index

The THI values to depict the level of heat stress are depicted in Fig. 4.1. the THI values both inside and outside the shed in the morning are 69.9 and 73.5 respectively, while in the afternoon the values were 74.9 and 86.5 respectively. The THI index inside shed proved that the animals were not stressed, while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant ($P<0.01$). The THI values description are: the values 72 and less are considered comfortable; THI values from 75 to 78 are considered stressful and THI above 78 considered extreme distress (McDowell, 1972).

4.2 Real time amplification plot of target genes

Amplification plot showed distinct variation of log (ΔRn) for different genes against PCR cycle number depicted in fig 4.2a. There was no amplification in non-template control (NTC). Multicomponent plot also showed the difference between the amplified and the non-amplified genes based on the graph pattern using the SYBR green dye depicted in 4.2b. Different genes FSHR, LHR, ESTRα, COX-2, PGF2α, PGE2 showed different Tm in the melt curve graph is depicted in 4.2c. The melt curve showed that the PCR reaction is free from primer-dimer artifacts based on the clear distinct curve which was absent in NTC.

Fig. 4.1. Average temperature humidity index (THI) for the study period both inside and outside the shed



The THI values were calculated as per method described by McDowell (1972). Accordingly the formula used was THI = 0.72 (T_{db} + T_{wb}) + 40.6 where, T_{db} = Dry bulb temperature in °C; T_{wb} = Wet bulb temperature in °C. The THI values 72 and less are considered comfortable; THI values between 75-78 are considered stressful and THI above 78 considered extreme distress

Fig. 4.2a Description of amplification plot showing distinct variation of log (ΔRn) for different genes against PCR cycle number

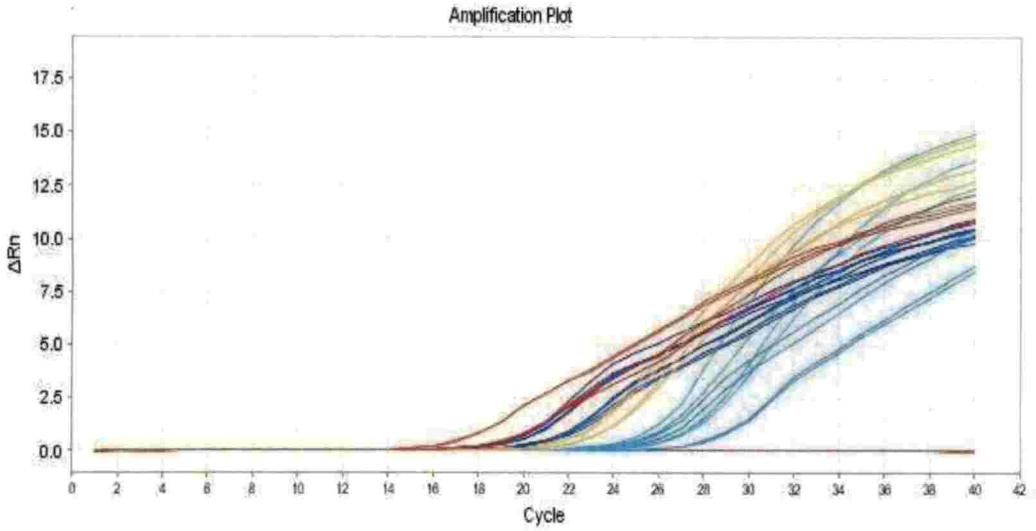


Fig. 4.2b Description of multicomponent plot also showing the difference between the amplified and the non-amplified genes based on the graph pattern using the SYBR green dye

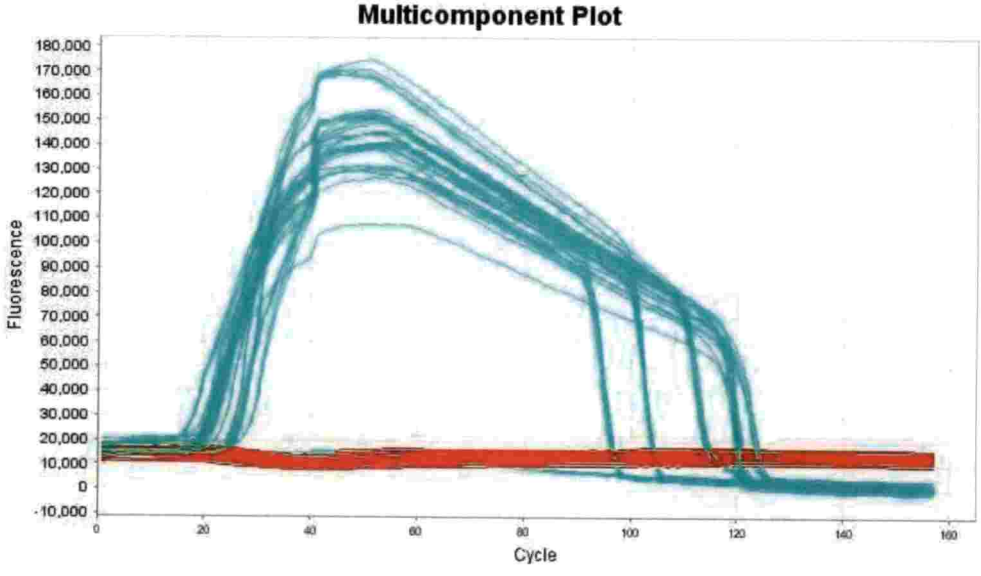
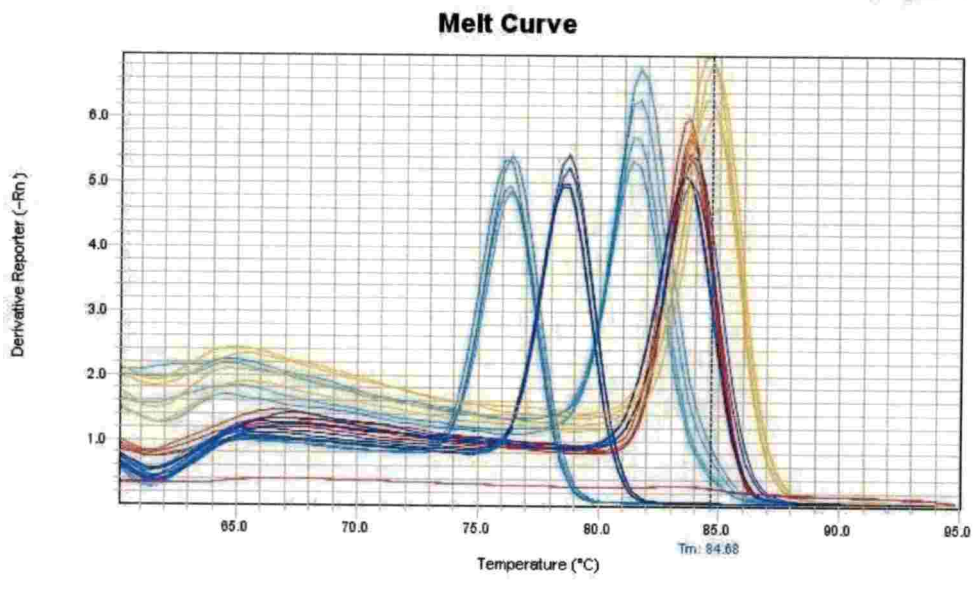


Fig. 4.2c Description of different reproduction related genes showing different Tm in the melt curve.



4.3. Follicle stimulating hormone receptor (FSHR) mRNA expression pattern

Fig. 4.3 describes the heat stress impact on the expression patterns of FSHR in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus FSHR in control and heat stress groups are 1.0 and 1.4 respectively. Although the FSHR gene expression in heat stress group showed the trend of up regulation as compared to the control group, still the differences between the groups were not statistically significant. Further, no correlation was established between THI and FSHR gene expression pattern (Table 4.1).

4.4. Relative luteinizing hormone receptor (LHR) mRNA expression pattern

Fig. 4.4 depicts the heat stress impact on the expression patterns of LHR in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus LHR in control and heat stress groups are 1.0 and 0.87 respectively. Although the LHR gene expression in heat stress group showed trends of down regulation as compared to the control group, still the differences between the groups were not statistically significant. Further, no significant correlation was established between THI and LHR gene expression pattern (Table 4.1).

4.5. Relative estrogen receptor alpha (ESTRα) mRNA expression pattern

Fig. 4.5 describes the heat stress impact on the expression patterns of ESTRα in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus ESTRα in control and heat stress groups are 1.0 and 10.5 respectively. Further, it was evident that the ESTRα expression pattern was significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. Further, no significant correlation was established between THI and ESTRα gene expression pattern (Table 4.1).

Fig. 4.3. Relative quantitative expression patterns of uterus FSHR mRNA in both control and heat stressed Malabari goats

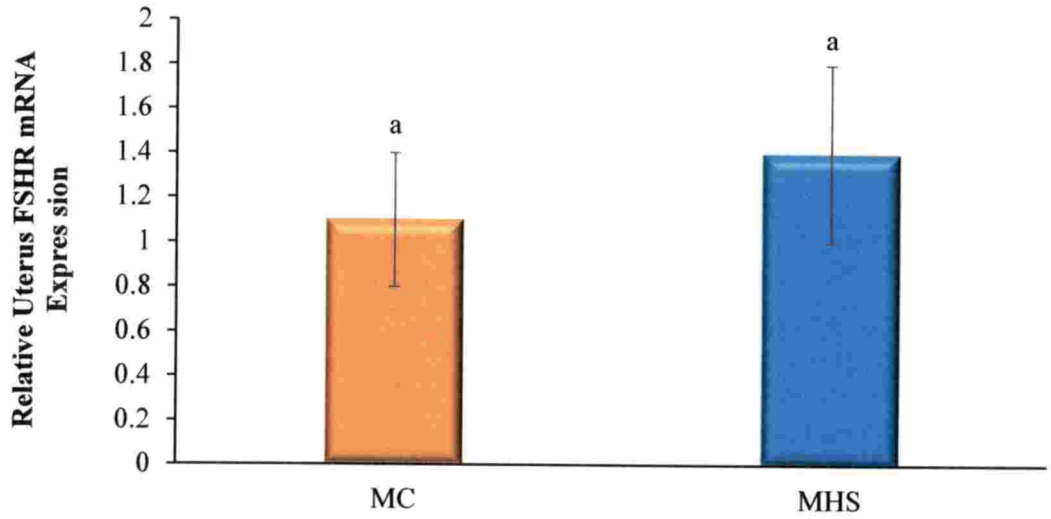
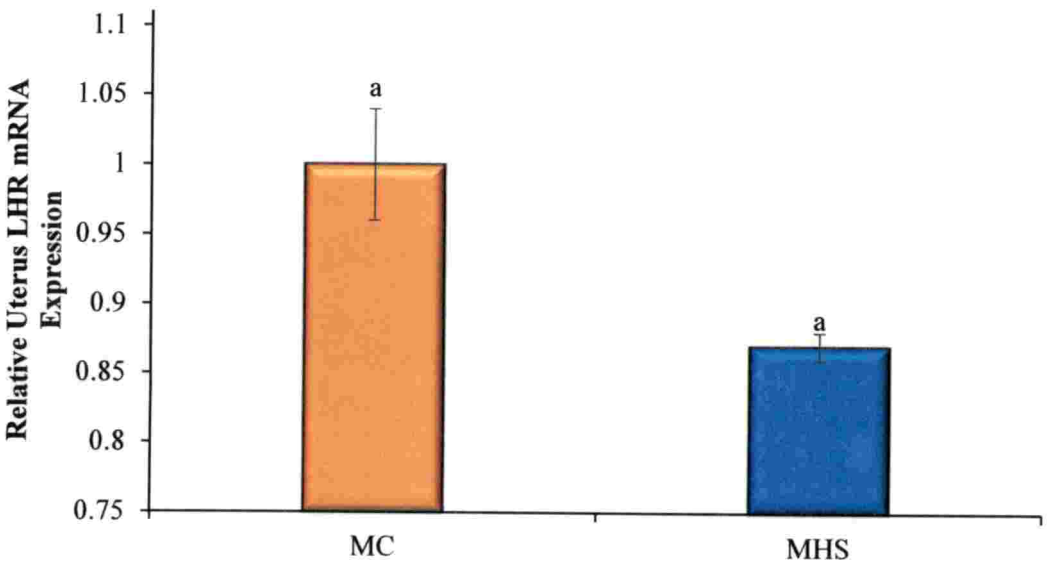


Fig. 4.4. Relative quantitative expression patterns of uterus LHR mRNA in both control and heat stressed Malabari goats



4.6. Relative cyclooxygenase-2 (COX-2) mRNA expression pattern

Fig. 4.6 illustrates the heat stress impact on the expression patterns of COX-2 in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus COX-2 in control and heat stress groups are observed as 1.0 and 0.65 respectively. Even though the COX-2 gene expression in heat stress group showed similar trend of down regulation as compared to the control group but the differences between the groups were not statistically significant. Further, no significant correlation was established between THI and COX-2 gene expression pattern (Table 4.1).

4.7. Relative prostaglandin F2 alpha (PGF2α) mRNA expression pattern

Fig. 4.7 depicts the heat stress impact on the expression patterns of PGF2α in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus PGF2α in control and heat stress groups are 1.0 and 2.78 respectively. Further, it was evident that the PGF2α expression pattern was significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. Further, a strong positive correlation ($P<0.01$) was established between THI and PGF2α gene expression pattern (Table 4.1).

4.8. Relative prostaglandin E2 (PGE2) mRNA expression pattern

Fig. 4.8 depicts the heat stress impact on the expression patterns of PGE2 in both control and heat stress groups of Malabari breed. The fold changes in expression pattern of uterus PGF2 in control and heat stress groups are 1.0 and 5.9 respectively. Further, it was evident that the PGE2 expression pattern was significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. Further, a strong positive correlation ($P<0.01$) was established between THI and PGE2 gene expression pattern (Table 4.1).

Fig. 4.5 Relative quantitative expression patterns of uterus ESTRα mRNA in both control and heat stressed Malabari goats

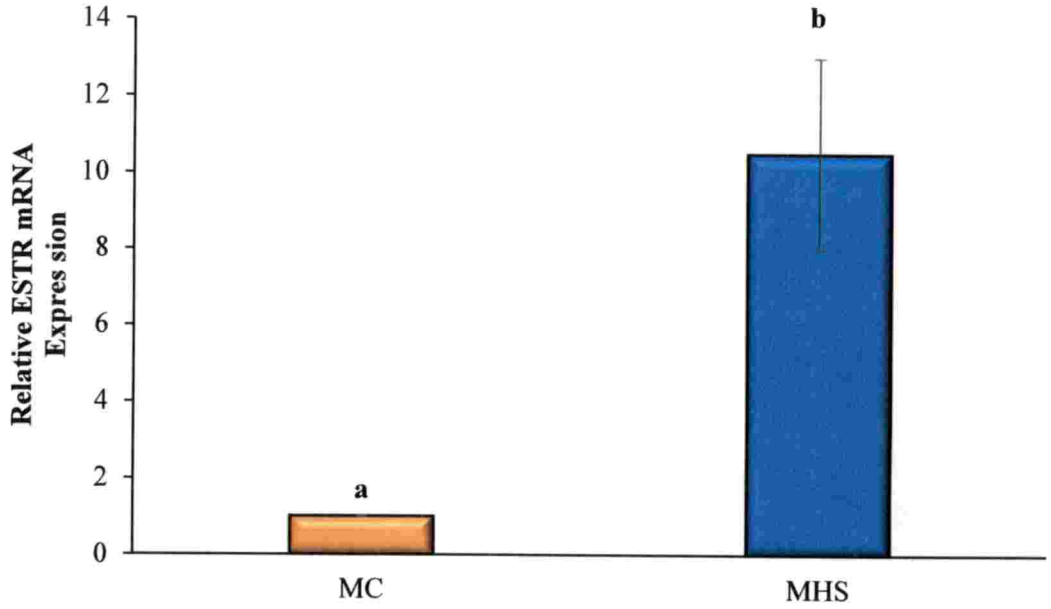


Fig. 4.6. Relative quantitative expression patterns of uterus COX2 mRNA in both control and heat stressed Malabari goats

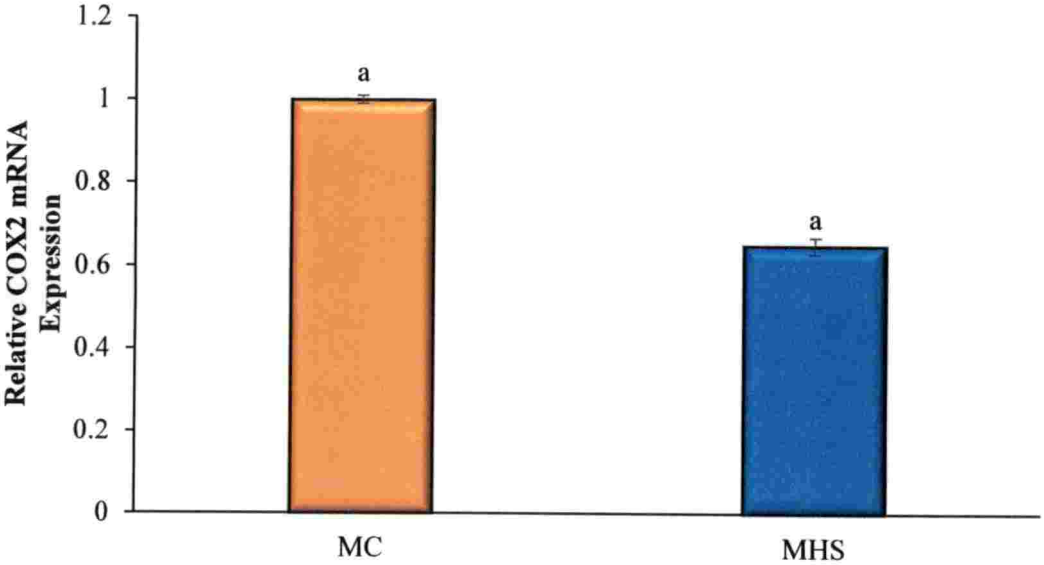


Fig. 4.7. Relative quantitative expression patterns of uterus PGF2α mRNA in both control and heat stressed Malabari goats

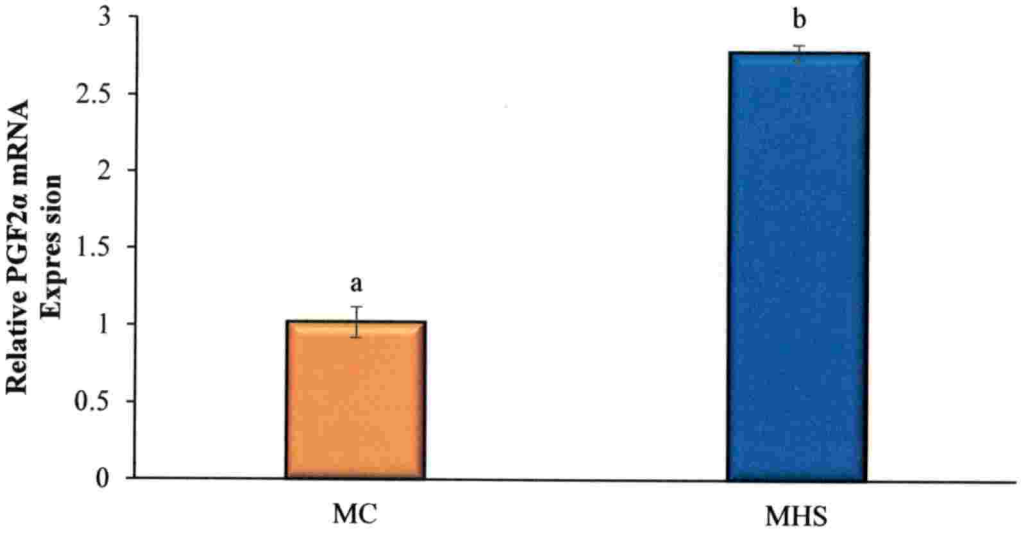
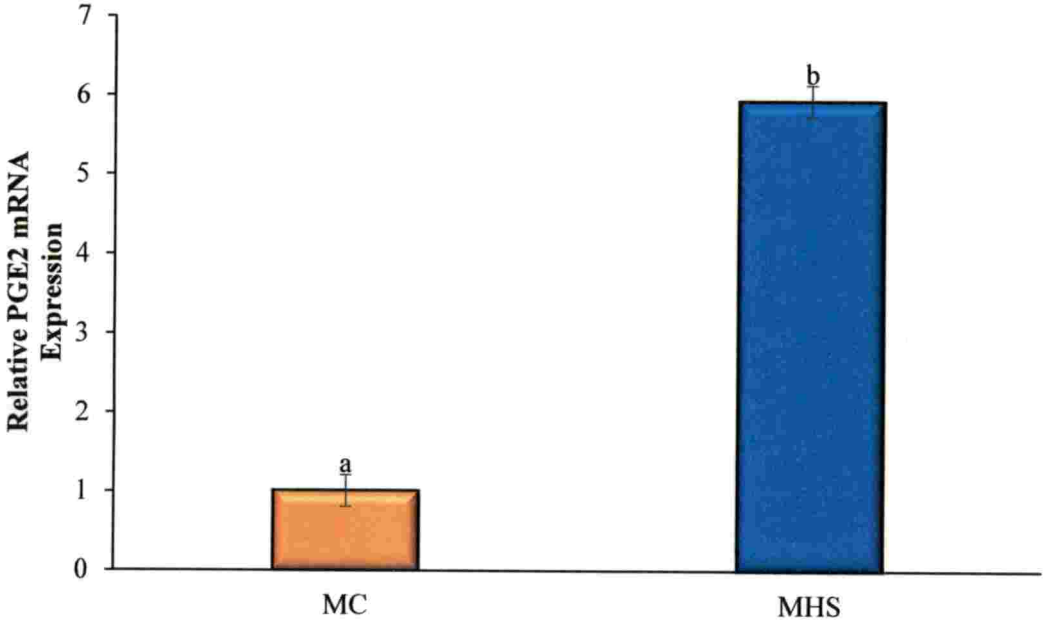


Fig. 4.8. Relative quantitative expression patterns of uterus PGE2 mRNA in both control and heat stressed Malabari goats



4.9. Histological section of uterus

4.9.1 Uterine Changes

The histopathological changes in uterus between MC and MHS groups were depicted in Fig. 4.9. The epithelial cells of endometrial villi of MHS group showed degenerative changes ($P<0.01$) with less differentiation compared to MC group. The functional endometrial glands were less in number in MHS group ($P<0.01$) as per field of observation. There were no significant changes ($P>0.05$) observed in myometrium and perimetrium between MC and MHS groups.

Fig. 4.9 represents the histopathological changes in uterus between MC and MHS groups

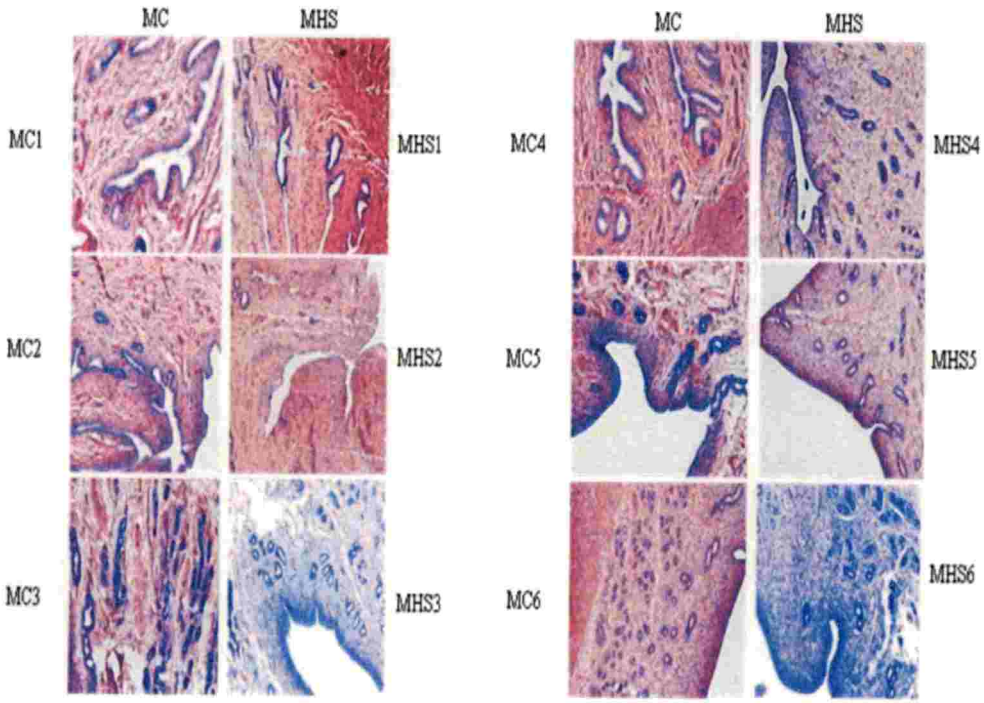


Table 4.1. Correlation association between THI and reproduction related gene expression

	THI	FSHR	LHR	ESTR	COX2	PGF2α	PGE2
THI	1						
FSHR	0.25	1					
LHR	-0.77	-0.58	1				
ESTR	0.79	-0.24	-0.58	1			
COX2	-0.51	-0.39	0.90*	-0.55	1		
PGF2α	0.98**	0.08	-0.65	0.84*	-0.40	1	
PGE2	0.99**	0.17	-0.73	0.82*	-0.49	0.99**	1

THI- Temperature humidity index; FSHR- Follicle stimulation hormone receptor; LHR- Luteinizing hormone receptor; ESTR- Estrogen receptor; COX-2- Cyclooxygenase 2; PGF2α- Prostaglandin F2 alpha; PGE2- Prostaglandin E2

**Indicates statistical significance at $P < 0.01$; * Indicates statistical significance at $P < 0.05$

DISCUSSION

CHAPTER 5

DISCUSSION

Heat stress seems to be the most critical factor negatively influencing the reproductive performance of domestic livestock (Sejian *et al.*, 2014; Al-Dawood *et al.*, 2017; Polsky *et al.*, 2017). Among the ruminant species, small ruminants and in particular, goat seems to be most resilience species to withstand the heat stress impact and maintain their productive potential (Silanikove, 2000). Most of the indigenous goat breeds are well known for their survival in their native tract and mostly the non-descript and other indigenous goat breeds are distributed in the tropical regions where generally adverse environmental condition persists which are not congenial for maintaining the productive performance. Malabari breed goat is one such breed in Southern India well known for its adaptive capability in hot humid tropical environment (Pragna *et al.*, 2017). Although there are several reports which established the adverse impacts of heat stress on the reproductive performance of goats, studies pertaining to heat stress influence on hypothalamic-pituitary-gonadal (HPG) axis associated genetic traits are very meagre. Therefore, the results obtained from the current study on the establishment of heat stress associated changes on the expression patterns of the various HPG axis genes is the first of its kind in domestic animals. This signifies the importance of this study to elucidate the molecular mechanisms associated with heat stress influenced reproductive performance in goats.

The THI index followed in the study clearly established the heat stress for the animals as any cumulative value above 75 as per McDowell (1972) model was considered to indicate extremely severe heat stress to animals.

5.1. Expression patterns of different reproduction related genes

5.1.1. Follicle Stimulating Hormone Receptor (FSHR)

The expression pattern of FSHR mRNA did not differ between the MC and MHS groups. It is a general finding that FSH secretion is elevated under heat stress condition probably due to reduced inhibition of negative feedback from smaller follicles, which ultimately affect the reproductive efficiency of dairy animals (Khodaei-Motlagh *et al.*, 2011). Further, it was observed that heat stress suppressed equine chorionic gonadotrophin (eCG)-induced follicular growth and caused a significant reduction of FSHR content in the granulosa cells in rats (Shimizu *et al.*, 2000; Ozawa *et al.*, 2005). The heat stress induced reduced FSHR expression could culminate in the lack of sufficient FSH stimulation to increase the aromatase activities leading to reduced estradiol production reflecting the poor reproductive performance of the animals (Ozawa *et al.*, 2005). However in the current study, similar expression of FSHR was noticed in MHS group as compared to MC group. Generally more FSHR expression was recorded in the prolific goat (Abdennebi *et al.*, 1999; Cui *et al.*, 2009). Malabari being a prolific breed was able to mount a better FSHR response. The comparable levels of FSHR mRNA expression between MC and MHS groups indicate the extreme adaptive nature of this breed for the heat stress challenges. Further, FSHR was also correlated with greater ovulation rate in prolific breeds and was found to be associated with greater gonadotropin responsiveness during the early follicular phase (Scaramuzzi *et al.*, 1993; Abdennebi *et al.*, 1999; Cui *et al.*, 2009). The comparable FSHR expression in both the groups of animals indicates the non-compromised ovulation rate in Malabari breed during heat stress exposure. Further, the non-significant correlation between THI and FSHR indicated that heat stress was not able to induce changes in the expression pattern of this gene in Malabari breed.

5.1.2. Luteinizing Hormone Receptor (LHR)

The LHR mRNA expression pattern also was similar between MHS and MC groups. This indicates that heat stress did not alter the expression pattern of LHR in Malabari goats. However, there are reports suggesting the lower LHR in heat stressed goats (Ozawa *et al.*, 2005; Krishnan *et al.*, 2017). This was attributed to the altered follicular functions during heat stress, including follicular dynamics and steroidogenic activity and this has been described as a major factor in inducing reduced summer fertility (Wolfenson *et al.*, 1995; Ozawa *et al.*, 2005; Das *et al.*, 2016). The heat stress induced reduced LHR could be the reason for suppressed follicular responsiveness to LH. This culminates in lack of sufficient LH stimulation leading to regression of the follicles before ovulation thus resulting in the reduced reproductive performance (Takahashi, 2012).The non-significant difference in LHR expression pattern between MC and MHS groups in the current study reflects the appropriate ovulation in these heat stressed animals, which could be again attributed to the extreme adaptive nature of this breed to heat stress. Further, the non-significant correlation between THI and LHR indicated that heat stress was not able to induce changes in the expression pattern of this gene in Malabari breed.

5.1.3 Estrogen Receptor Alpha (ESTRα)

The ESTRα expression was higher in MHS group as compared to MC group. There are no reports available on the influence of heat stress on ESTRα expression in ruminant livestock. Therefore, the current study is the first of its kind to establish the higher expression pattern of ESTRα in MHS group. The ESTRα was found to be the predominant receptor subtype controlling the reproductive performance in animals (Binelli *et al.*, 2018). The very high expression of this receptor in heat stressed goats signifies the importance of this receptor in controlling the reproductive activities of Malabari goats. The significantly higher expression pattern of ESTRα gene in MHS group goats could indicate the non-utilization of these receptors and this could be

attributed to the significantly lower estradiol concentration in heat stressed animals (Sejian *et al.*, 2011; Ozawa *et al.*, 2005; Roth, 2008). Therefore, higher expression of uterine endometrial ESTRα gene could serve as an ideal indicator for heat stress impact on reproductive performance in Malabari goats.

5.1.4 Cyclooxygenase 2 (COX-2)

COX-2 was considered developmentally important gene that transcribes an enzyme related to prostaglandin synthesis (Silva *et al.*, 2013). A similar mRNA expression pattern of COX-2 gene between MC and MHS groups was established in the study. El-Sayed *et al.* (2006) reported significantly lower COX-2 gene expression in Jersey cows while found no effect on the expression pattern of COX-2 in heat stressed Nellore cow. This difference in expression pattern of COX-2 gene between Jersey and Nellore breeds of cattle could be attributed to the breed differences. Further, the non-significant influence of heat stress on COX-2 gene expression could be attributed to its indigenous nature well known for its survival in tropical environment. The same reason could be speculated for the no effect of heat stress on COX-2 expression pattern in Malabari breed goats well known for its survival in hot and humid tropical environment. Hence, the non-significant variation in COX-2 gene expression pattern between the control and heat stress group of Malabari goats indicates the extreme adaptive nature of this breed in maintaining prostaglandin production.

5.1.5 Prostaglandin F2 α (PGF2α)

Prostaglandins (PGs) produced by endometrium serves as a crucial mediators in maternal recognition of pregnancy, implantation and parturition (Poyser, 1995). The PGF2α mRNA expression was significantly higher in MHS group as compared to MC group. Exposure of heat stress in the present study resulted in marked increase in the expression pattern of PGF2α from the endometrium. Similar finding of heat stress

induced increase in production of PGF2α in endometrium was reported in dairy cow (Putney *et al.*, 1988) and sheep (Sukanta *et al.*, 2017). However, Kobayashi *et al.* (2013) reported contrasting result of no effect of temperature stress on PGF2α production. Increased PGF2α synthetic capacity of endometrium exposed to heat stress may be due to heat-induced alterations in endometrial cellular membranes resulting in increased mobilization of substrate for prostaglandin biosynthesis (Sukanta *et al.*, 2017). As maintenance of luteal function is associated with alterations in endometrial prostaglandin production and increased prostaglandin secretion following heat stress may compromise corpus luteum (CL) function and initiate luteal regression (Thatcher *et al.*, 1984).

5.1.6 Prostaglandin E2 (PGE2)

Heat stress may be a cause of summer infertility by decreasing the oviductal motility via promoting PGE2 production (Kobayashi *et al.*, 2013). Thermal exposure significantly increased the expression pattern of PGE2 in the endometrial cells of MHS group as compared to MC group goats. Similarly, Kobayashi *et al.* (2013) also reported increased PGE2 production in the heat stressed dairy cow. This heat stress induced increased PGE2 production could be due to either increases in the expressions of PGESs or increases in the PGES activity. However, Sukanta *et al.* (2017) did not observe any difference in the expression pattern of PGE2 in sheep endometrium. Our results agree with the report of Putney *et al.* (1988) and Malayer *et al.* (1999) in dairy cows wherein they suggested that secretion of both PGF2α and PGE2 in endometrium increased in response to heat stress. Further, a strong positive correlation of THI with both PGF2α and PGE2 justifies the above argument of increased expression of these prostaglandin genes to be detrimental to the reproductive performance of Malabari breed goat.

5.1.7 Histopathological section of uterus

The effect of heat stress induced changes in the histological section of uterus between MC and MHS groups also was similar to the earlier discussion of significant influence of heat stress on uterus related ESTRα, PGF2α and PGE2. This shows that the reproductive activities of Malabari goats were very sensitive at uterine level and not at the hypothalamus and pituitary level of the HPG activities.

SUMMARY AND CONCLUSION

CHAPTER 4

SUMMARY AND CONCLUSION

Heat stress is the major factor which negatively influences the reproductive performance of ruminant livestock. Malabari breed of goat is an indigenous breed well known for its survival in harsh environmental condition. Although there are several reports, which established the adverse impacts of heat stress on the reproductive performance of goats, studies pertaining to heat stress influence on hypothalamic-pituitary-gonadal (HPG) axis associated genetic traits are very meagre. Therefore, the study was conducted with the primary objective of establishing the effect of heat stress on expression patters of different traits that controls Malabari goat reproduction.

The study was conducted for a period of 45 days. Twelve animals were used in this study. The animals were randomly allocated into two groups of six animals each, MC (n=6; Malabari control), and MHS (n=6; Malabari heat stress). The MC animals were maintained in the shed in thermo-neutral condition while MHS animals were exposed outside to summer heat stress between 10:00 h to 16:00 h during the experimental period. The MC animals were fed and watered inside the shed, while MHS animals were fed and watered with exposure to summer heat stress in the outside environment. The animals were slaughtered at the end of the study and their uterine samples were collected for gene expression study.

The THI inside shed proved that the animals were not stressed while in the outside environment they were extremely distressed. This difference in THI between inside and outside the shed were highly significant ($P<0.01$). The THI value of 86.5 recorded during outside exposure in MHC group clearly indicated that these animals were subjected to extremely severe heat stress.

The expression pattern of FSHR mRNA did not differ between the MC and MHS groups. Although the FSHR gene expression in heat stress group showed the trend of up regulation as compared to the control group, still the differences between

the groups were not statistically significant. Further, no correlation was established between THI and FSHR gene expression pattern. The comparable levels of FSHR mRNA expression between MC and MHS groups indicated the extreme adaptive nature of this breed for the heat stress challenges.

The LHR mRNA expression pattern also was similar between MHS and MC groups. This indicates that heat stress did not alter the expression pattern of LHR in Malabari goats. The non-significant difference in LHR expression pattern between MC and MHS groups in the current study reflected the appropriate ovulation in these heat stressed animals, which could be again attributed to the extreme adaptive nature of this breed to heat stress. Further, the non-significant correlation between THI and LHR indicated that heat stress was not able to induce changes in the expression pattern of this gene in Malabari breed.

The ESTRα expression pattern was significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. The current study is the first of its kind to establish the higher expression pattern of ESTRα in heat stressed goats. The very high expression of this receptor in heat stressed goats signifies the importance of this receptor in controlling the reproductive activities of Malabari goats. The significantly higher expression pattern of ESTRα gene in MHS group goats could indicate the non-utilization of these receptors and this could be attributed to the significantly lower estradiol concentration in heat stressed animals. Therefore, higher expression of uterine endometrial ESTRα gene could serve as an ideal indicator for heat stress impact on reproductive performance in Malabari goats.

A similar mRNA expression pattern of COX-2 gene between MC and MHS groups was established in the study. Even though the COX-2 gene expression in heat stress group showed similar trend of down regulation as compared to the control group but the differences between the groups were not statistically significant. Further, no significant correlation was established between THI and COX-2 gene expression pattern. The no effect of heat stress on COX-2 expression pattern in Malabari breed goats could be attributed to the indigenous nature of this breed and

reflects the extreme adaptive nature of this breed in maintaining prostaglandin production.

The PGF2α expression pattern was significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. Further, a strong positive correlation ($P<0.01$) was established between THI and PGF2α gene expression pattern. Increased PGF2α synthetic capacity of endometrium exposed to heat stress may be due to heat-induced alterations in endometrial cellular membranes resulting in increased mobilization of substrate for prostaglandin biosynthesis.

Similarly, the PGE2 expression pattern was significantly ($P<0.05$) up regulated in heat stress group as compared to the control group animals. This heat stress induced increased PGE2 production could be due to either increase in the expressions of PGESs or increase in the PGES activity. Further, a strong positive correlation of THI with both PGF2α and PGE2 justifies the above argument of increased expression of these prostaglandin genes to be detrimental to the reproductive performance of Malabari breed goat.

The epithelial cells of endometrial villi of MHS group showed degenerative changes ($P<0.01$) with less differentiation compared to MC group. The functional endometrial glands were less in number in MHS group ($P<0.01$) per field of observation. The effect of heat stress induced changes in the histological section of uterus between MC and MHS groups also was similar to the earlier discussion of significant influence of heat stress on uterus related ESTRα, PGF2α and PGE2. This shows that the reproductive activities of Malabari goats were very sensitive at uterine level of the HPG activities. Therefore, the results obtained from the study on the establishment of heat stress associated changes on the expression patterns of the various HPG axis genes is the first of its kind in domestic animals. This signifies the importance of this study to elucidate the molecular mechanisms associated with heat stress influenced reproductive performance in goats. The study also indicated that ESTRα, PGF2α and PGE2 genes could serve as ideal indicators of heat stress effect on reproductive performance in indigenous Malabari goats.

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ABSTRACT

EFFECT OF HEAT STRESS ON THE EXPRESSION PATTERNS OF DIFFERENT REPRODUCTION RELATED GENES IN MALABARI GOATS

by

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ABSTRACT

Heat stress is the major factor which negatively influences the reproductive performance of ruminant livestock. Although there are several reports which established the adverse impacts of heat stress on the reproductive performance of goats, studies pertaining to heat stress influence on hypothalamic-pituitary-gonadal (HPG) axis associated genetic traits are very meagre. Therefore, the study was conducted with the primary objective of establishing the effect of heat stress on expression patters of different traits that controls Malabari goat reproduction. The study was conducted during 45 days using twelve 10 months to one year old Malabari goats. The goats were randomly allocated into two groups: MC (n=6; Malabari control) and MHS (n=6; Malabari heat stress). Goats were stall-fed with a diet composed of 60% roughage and 40% concentrate. All animals had access to *ad-libitum* feed and water and they were fed and watered individually. The MC goats were placed in the shaded pens while MHS goats were exposed to heat stress in outside environment between 10.00 h to 16.00 h. At the end of study period, all 12 animals were slaughtered and their uterus tissues were collected for gene expression and histopathological studies. The temperature humidity index (THI) inside shed (74.9) proved that the animals were not stressed while in the outside environment (86.5) the animals were extremely distressed. Heat stress significantly ($P<0.05$) influenced the expression patterns of estrogen receptor α (ESTRα), prostaglandin F2 α (PGF2α) and prostaglandin E2 (PGE2). Further, a strong positive correlation ($P<0.01$) was established for THI with both PGF2α and PGE2 gene expressions. However, heat stress did not influence the expression patterns of follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR) and cyclooxygenase-2 (COX-2) genes. Further, the non-significant correlation of THI with FSHR, LHR and COX-2 genes could be attributed to the resilience capacity of Malabari breed to heat stress. The histopathological section of uterine epithelial cells showed degenerative changes ($P<0.05$) with less differentiation in MHS group as compared to MC group. Therefore, the results obtained from the study on the establishment of heat stress associated changes on the expression patterns of the various HPG axis genes is the first of its kind in domestic animals. The results from the study clearly indicated that

heat stress was able to alter reproductive activity related gene expression at uterine level to reduce the reproductive efficiency in Malabari goats. The study also indicated that ESTRα, PGF2α and PGE2 genes could serve as ideal indicators of heat stress effect on reproductive performance in indigenous Malabari goats.

Keywords: Climate change; ESTRα; Heat stress; PGF2α; PGE2; Reproductive trait