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Research Article

Effect of Summer Heat Stress on Fertility and Blood Parameters in Ewes and Nanny Goats

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ABSTRACT

One hundred twenty-three (54 fertile and 69 infertile) ewes and nanny goats' enrolled in this study to evaluate the effect of heat stress on their reproductive performance. The data collected were ambient temperature, humidity, coat, skin color, weight, and body temperature. Blood samples (7 mL) drawn for complete blood analysis, and determination of serum cortisol and cardiac troponin I levels. Data are statistically analyzed via ANOVA. Results revealed significant ($P<0.05$) high values of ambient temperature and THI (44.88 ± 0.25 °C and 91.18 ± 0.27, respectively) recorded during summer. The body temperature was higher significantly ($P<0.05$) in the heat stressed ewes and nanny goats with wool, hair coat, white or colored skin than in the non-stressed animals. The ambient temperature °C, THI, and body temperature °C were significantly ($P<0.05$) higher in the heat stressed ewes and nanny goats (fertile and infertile) than the non-stressed animals. Serum cardiac troponin levels were significantly ($P<0.05$) higher in the infertile stressed than in the fertile non-stressed and stressed animals (95.97 ± 17.70 vs. 65.37 ± 14.50 and 73.43 ± 25.27 ng/mL, respectively). In conclusion, heat stress exerted a drastic effect on ewes and nanny goats' reproduction that appears in most blood parameters and cardiac troponin I levels.

Keywords: Small ruminants; Heat stress; Blood parameters; Cardiac troponin.

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INTRODUCTION

Sheep and goats are the major livestock species in the Kingdom of Saudi Arabia (KSA; 92% of total livestock), 17.5 million sheep and 6.1 million goats. Meat and milk are the primary products of these small ruminants that playing important role in the socio-economic wellbeing of the rural people in KSA (Ministry of Environment, Water & Agriculture, 2021). Several factors, including management procedures, nutrition, and environmental conditions affect livestock because they cause stress, which is defined as the inability of an animal to cope with its environment (Dobson et al., 2012). Ambient conditions (temperature, relative humidity, solar radiation, and wind speed) are among the main abiotic factors that negatively affect livestock production and animal welfare because they induce heat stress under certain circumstances (Sejian, 2013). The summer in Saudi Arabia is characterized by high ambient temperature reaches >50°C, and low relative humidity. Therefore, farm animals raised in such sever climatic stress for almost 6 months of the year and become uncomfortable. Climate change is a major global threat to the sustainability of livestock systems. Continuous exposure of the animals to heat stress compromises growth, milk and meat production, reproduction and resistance to diseases and parasites (McManus et al., 2020).

The capacity of an animal to mitigate effects of increased environmental temperature, without progressing into stress response, differs within and between species (Joy et al., 2020). Compared to other domestic species, small ruminants such as sheep are well adapted to extreme climate conditions, making them an option in arid and semi-arid regions with low forage resource availability (Shinde and Sejian, 2013). However, heat stressed sheep exhibit low fertility and fetal development and growth, as well as unsuitable weight gain and feed efficiency during the fattening period (Vicente-Pérez et al., 2015). The blood system is sensitive to temperature changes and is an important indicator of physiological responses to stressors. Several factors such as species, breed, sex, age, nutrition, diseases, physiological stage and seasonal variations can affect the pattern of hematological values (Al-Eissa et al., 2012). Some enzymes, especially alanine aminotransferase (ALT), aspartate aminotransferase (AST) are good indicators of serum protein deficiency which is a result of acute and chronic liver disease (Stojević et al., 2008). The enzyme ALT is normally found in hepatocytes only. However, AST present in hepatocytes, red blood cells, and in cardiac and skeletal muscles. Those enzymes are considered as biomarkers of hepatic affection (Evans, 2009). Audet et al. (2015) stated that circulating levels of cardiac troponin (cTnI), a well-established clinical biomarker of cardiovascular damage could serve as a robust biomarker of heat stroke severity in rats. In sheep, plasma glucose was lower while blood urea nitrogen (BUN) was higher in heat stress as compared with control ewes (Indu et al., 2014). In response to various stresses, the hypothalamic pituitary-adrenocortical (HPA) system is activated, which induces the secretion of cortisol into the blood (Dhama et al., 2019).

The present study aimed to understanding the impact of climatic stress on ewes and nanny goats' reproductive performance.

MATERIALS AND METHODS

This study approved ethically by the Deanship of Scientific Research (DSR), Research Ethics Committee (REC), King Faisal University. REC REF NUMBER "KFU-REC/2021-04-06".

Experimental animals

Experimental animals (n = 132) were selected to determine the impact of climate on reproductive performance. Fertile animals (n = 54) included 32 normal cyclic "7 ewes and 25 goats", 7 pregnant "4 ewes and 13 goats", and 5 parturient "3 ewes and 2 goats". Infertile females (n = 69) were 34 repeat breeders "28 ewes and 6 goats", 24 had endometritis "12 ewes and 12 goats", 5 with puerperal infection "4 ewes and one goat", 4 goats had ovarian cyst, and 2 with anestrus "one ewe and one goat".

These animals presented in random open farms in Al-hofuf, Saudi Arabia (25°23′N 49°35′E / 25.383°N 49.583°E / 25.383; 49.583), with temperature ranged from 22°C in December to 46°C in July and August, and humidity from 22% in June to 57% in December with an average 39% throughout the year. They fed on normal ration (berseem and concentrates) and received human management care (feeding, ventilation, exercise and cleaning) all over the period of study. There were no exclusion factors that underlying health issues of these animals, like diet and management practices. Data about these animals were collected that include ambient temperature (°C), humidity (%; https://www.timeanddate.com/weather/saudi-arabia/hofuf/ext), and temperature humidity index (THI), body coat, skin color, weight (Kg), and body temperature (°C).

THI was calculated according to the following equation (Marai et al., 2001)

$$THI = 0.8\ T + RH\ (T-14.4) + 46.4$$

where T = ambient or dry-bulb temperature in °C and RH=relative humidity expressed as a proportion i.e., 75% humidity is expressed as 0.75.

Blood samples and analyses

Seven ml of blood was drawn into two vacutainer tubes, one contains EDTA that used immediately for complete blood analysis, and the other plane tube centrifuged at 4000g for 15 min. and the separated serum stored at -80°C pending analyses. Serum was used for biochemical analyses of procalcitonin (PCT; ng/mL), glucose (GLU; mg/dL), creatine phosphokinase (CPK; U/L), sodium (Na; mmol/L), potassium (K; mmol/L), chloride (Cl; mmol/L), magnesium (Mg; mg/dL), urea (mg/dL), and blood ammonia (BA; µmol/L) using chemistry analyzer, large animal panel machine, product code 900-170, chem c, Skyla corporation®. Serum cortisol and cardiac troponin I were determined by ELISA (Absorbance Microplate Reader ELx 800TM BioTek®, USA; Microplate Strip Washer ELx 50TM BioTek®, USA) using commercial kits (SinoGeneclon Biotech Co., Ltd, China). The intra and inter assay CVs were (CV<8%, CV<10%) and (CV<8%, CV<10%) for both Cortisol and cardiac troponin I, respectively.

Statistical analysis

The data of ewes and nanny goats (Table 1) was divided according to seasons, ambient temperature °C, humidity %, bread (sheep and goat), age of the female, coat (wool and hair), skin color, body weight, and body temperature °C. Data are presented as mean ± standard error of the mean (mean±SEM) for the previous parameters and calculated by analysis of variance (ANOVA) using commercial software (GraphPad Prism 9, 2022). The mean of the variables measured for groups was compared using Tukey post hoc test. P-value less than 0.05 (P<0.05) was taken as statistically significant.

Table 1. The data division of ewes and nanny goats.

Seasons	Autumn (September – November) Winter (December – February) Spring (March – May) Summer (June – August)
Breed	Sheep Goat
Age	A (1.5 – 3.5 years) B (4.0 – 5.5 years) C (6.0 – 9.0 years)
Coat	Wool Hair
Skin color	White colored

RESULTS

As shown in Table 2, a significantly (P<0.05) high differences in values of ambient temperature, humidity %, and THI between the non-stressed and stressed animals. In Table 3, the ambient temperature °C, THI, and body temperature °C were significantly (P<0.05) higher in the heat stressed small ruminants (fertile and infertile) than the non-stressed animals. However, the percentage humidity was significantly (P<0.05) higher in the non-stressed small ruminants (fertile and infertile) than in the heat stressed animals.

Table 2. The effect of ambient temperature, humidity % and THI on ewes and nanny goats (Mean±SEM)

Weather parameters	Non-stressed animals (n=43)	Stressed animals (n=80)
Ambient temp. (ºC)	22.26^{a} ± 1.36	44.51^{b} ± 0.29
Humidity %	44.05^{a} ± 3.56	11.13^{b} ± 0.43
*THI	66.48^{a} ± 1.48	90.74^{b} ± 0.32

Means with dissimilar superscripts in the same row are significantly different at P<0.05

*Temperature-Humidity Index

Table 4 showed a significant (P<0.05) difference in monocytes (%) and red blood corpuscles (m/µL) between the fertile stressed animals and infertile non-stressed or stressed ewes and nanny goats. There was a significant (P<0.05) difference in hematocrit (%) and RDWs (fL) between the fertile stressed animals and infertile non-stressed small ruminants. The values of MCV (fL) and MCH (pg) were significantly (P<0.05) lower in the fertile stressed animals than in the infertile stressed or non-stressed ewes and nanny goats.

There was a high significant (P<0.01) difference in platelet count ($x10^3$/µL) between the infertile non-stressed and infertile stressed ewes and nanny goats. There was a significant (P<0.05) difference in mean platelet volume (fL) between the infertile stressed and both fertile non-stressed and stressed animals.

In Table 5, the reading of procalcitonin (ng/mL) was significantly (P<0.05) higher in the fertile non-stressed than in the infertile stressed animals. A highly significant (P<0.001) difference recorded in serum glucose (mg/dL) between the fertile non-stressed and both infertile non-stressed and stressed small ruminants. There was a significant (P<0.05) difference in serum creatine phosphokinase (U/L) between the infertile non-stressed and fertile non-stressed animals (Table 5).

Table 3. The effect of ambient temperature, humidity %, THI and body temperature on fertility in ewes and nanny goats (Mean±SEM)

Parameters	Fertile non-stressed (n=32)	Fertile stressed (n=22)	Infertile non-stressed (n=11)	Infertile stressed (n=58)
Ambient temp. (ºC)	$19.66^{a} \pm 1.58$	$44.91^{b} \pm 0.55$	$29.82^{c} \pm 0.30$	$44.36^{b} \pm 0.34$
Humidity %	$45.28^{a} \pm 3.60$	$9.86^{b} \pm 0.52$	$40.45^{a} \pm 8.38$	$11.60^{b} \pm 0.55$
THI (Marai et al., 2001)	$63.65^{a} \pm 1.72$	$91.17^{b} \pm 0.60$	$74.73^{c} \pm 0.32$	$90.58^{b} \pm 0.37$
Body temp. (ºC)	$38.04^{a} \pm 0.13$	$39.08^{b} \pm 0.19$	$37.52^{a} \pm 0.27$	$39.66^{c} \pm 0.10$

Means with dissimilar superscripts in the same row are significantly different at P<0.05

Table 4. The effect of heat stress on hematology and fertility in ewes and nanny goats (Mean±SEM)

Blood parameters	Fertile non-stressed (n=32)	Fertile stressed (n=17)	Infertile non-stressed (n=11)	Infertile stressed (n=49)
Monocytes %	$0.77^{ab} \pm 0.07$	$1.04^{a} \pm 0.14$	$0.51^{b} \pm 0.01$	$0.67^{b} \pm 0.05$
Red Blood Corpuscles (RBCs) m/µL	$14.58^{ad} \pm 0.59$	$16.06^{a} \pm 0.67$	$11.27^{bc} \pm 0.57$	$12.86^{cd} \pm 0.54$
Hematocrit (HCT) %	$27.11^{ab} \pm 0.73$	$23.89^{a} \pm 0.93$	$28.76^{b} \pm 0.62$	$25.66^{ab} \pm 0.65$
Mean Corpuscular Volume (MCV) fL	$19.25^{ab} \pm 1.14$	$15.94^{a} \pm 1.06$	$23.55^{b} \pm 2.10$	$21.49^{b} \pm 1.02$
Mean Corpuscular Hemoglobin (MCH) pg	$7.79^{ab} \pm 0.46$	$5.98^{a} \pm 0.29$	$9.14^{b} \pm 0.66$	$8.01^{b} \pm 0.36$
Red Cell Distribution Width standard deviation (RDWs) fL	$23.68^{ab} \pm 0.65$	$21.67^{a} \pm 0.78$	$26.07^{b} \pm 0.74$	$23.85^{ab} \pm 0.53$
Platelet count (PLT) $x10^{3}$/ µL	$243.80^{ab} \pm 38.42$	$216.25^{ab} \pm 37.61$	$328.70^{a} \pm 43.87$	$141.89^{b} \pm 23.24$
Mean Platelet Volume (MPV) fL	$5.15^{a} \pm 0.19$	$4.85^{a} \pm 0.17$	$5.42^{ac} \pm 0.17$	$5.74^{bc} \pm 0.12$

Means with dissimilar superscripts in the same row are significantly different at P<0.05.

Table 5. The effect of heat stress on blood chemistry and fertility in ewes and nanny goats (Mean±SEM).

Blood parameters	Fertile non-stressed (n=32)	Fertile stressed (n=17)	Infertile non-stressed (n=11)	Infertile stressed (n=49)
Procalcitonin (PCT) ng/mL	$0.11^{a} \pm 0.02$	$0.04^{ab} \pm 0.01$	$0.11^{ab} \pm 0.02$	$0.06^{b} \pm 0.01$
Glucose (GLU) mg/dL	$62.32^{a} \pm 3.12$	$52.11^{ac} \pm 3.68$	$34.60^{bc} \pm 1.90$	$41.45^{bc} \pm 2.02$
Creatine phosphokinase (CPK) U/L	$95.21^{a} \pm 13.59$	$141.33^{ab} \pm 37.82$	$251.40^{b} \pm 79.51$	$148.47^{ab} \pm 14.08$
Sodium (Na) mmol/L	$141.00^{ac} \pm 1.25$	$143.56^{bc} \pm 1.70$	$147.83^{b} \pm 4.23$	$145.14^{b} \pm 0.68$
Potassium (K) mmol/L	$5.57^{a} \pm 0.10$	$6.41^{ab} \pm 0.27$	$6.07^{ab} \pm 0.50$	$6.40^{b} \pm 0.13$
Sodium –Potassium ratio (Na-K)	$25.42^{ab} \pm 0.57$	$22.78^{a} \pm 0.46$	$27.67^{b} \pm 3.36$	$24.09^{ab} \pm 0.44$
Chloride (Cl) mmol/L	$107.47^{a} \pm 1.09$	$111.00^{ab} \pm 2.47$	$114.17^{ab} \pm 2.92$	$112.71^{b} \pm 0.73$
Magnesium (Mg) mg/dL	$1.96^{a} \pm 0.07$	$2.36^{ab} \pm 0.16$	$2.14^{ab} \pm 0.20$	2.41b ± 0.07
Urea mg/dL	$34.75^{ab} \pm 3.30$	$43.04^{a} \pm 3.08$	$45.90^{a} \pm 4.90$	32.00b ± 1.78
Blood ammonia (BA) µmol/L	$21.60^{a} \pm 5.22$	$43.16^{ab} \pm 10.59$	$70.03^{b} \pm 7.32$	$50.24^{ab} \pm 6.86$
Cortisol ngl/mL	203.52 ± 30.96	218.32 ± 29.54	171.62 ± 20.04	260.37 ± 14.74
Cardiac Troponin (cTnI) ng/mL	$13.07^{a} \pm 2.90$	$14.69^{a} \pm 5.05$	$15.20^{ab} \pm 1.94$	$19.19^{b} \pm 3.54$

Means with dissimilar superscripts in the same row are significantly different at P<0.05

There was a significant (P<0.05) difference in serum sodium (mmol/L) between the fertile non-stressed and infertile non-stressed and stressed ewes and nanny goats. The serum readings of potassium (K, mmol/L), chloride (Cl,

mmol/L), and magnesium (Mg, mg/dL) were significantly (P<0.01) higher in the fertile non-stressed than in the infertile stressed animals. The Na-K ratio was significantly (P<0.05) lower in the fertile stressed than in the infertile non-stressed ewes and nanny goats. The urea (mg/dL) serum level was significantly (P<0.05) higher in the fertile stressed and infertile non-stressed than in the infertile stressed animals. The blood ammonia levels (µmol/L) was significantly (P<0.01) lower in the fertile non-stressed than in the infertile non-stressed animals. The serum cardiac troponin levels (ng/mL) were significantly (P<0.01) higher in the infertile stressed than in the fertile non-stressed and stressed animals (Table 5).

DISCUSSION

High ambient temperature directly affects the reproductive performance of animals and impairs follicular development and oocyte maturation, and ovulation through the hypothalamic-pituitary-gonadal axis by inhibiting gonadotropin-releasing hormone (GnRH) in the hypothalamus (Servili et al., 2020). This inhibition in GnRH altering luteinizing and follicle-stimulating hormones' secretion and dynamics during the estrous cycle (La Salles et al., 2017). Heat stress induced a marked increase in the incidence of ruptured oolemma, cracked zona pellucida and a small number of fertilized ova (Casu et al., 1991). In the present study, heat stress exerted a drastic effect on body temperature, blood parameters, and fertility of stressed animals. Besides, high differences in values of ambient temperature, humidity %, THI, and body temperature found between the non-stressed and stressed animals. Similarly, high values of ambient temperature and relative humidity negatively affect livestock because they induce heat stress that increased ewes' body temperature 1.13 °C (Romo-Barron et al., 2019). Growth, milk production and reproduction are impaired under heat stress as a result of the drastic changes in biological functions caused by heat stress (Baumgard and Rhoads, 2013). Under thermoneutral condition sheep can keep their body temperature in a normal range utilizing sensible heat loss (Convection, conduction and radiation) to dissipate body heat to the surrounding environment (Al-Haidary, 2004).

Increased body temperature is the most important sign for heat stress in sheep (Al-Haidary, 2004) and goat (Alam et al., 2011). Even a rise of 1°C or less in rectal temperature is enough to reduce reproductive performance in most livestock species (Gupta and Mondal, 2021).

As shown in this study, the ambient temperature, THI, and body temperature were higher in the heat stressed ewes and nanny goats (fertile and infertile) than the non-stressed animals. On the same direction, the animal reproductive system is more sensitive to temperature than other body parts (Gupta and Mondal, 2021). Heat stressed sheep exhibit low fertility through the reduction in expression of estrus (80% unnoticeable estrus during summer), ovulation, fertilization, conception rate, fetal development and pregnancy rate, and increase embryo loss by 71.4% (Gupta and Mondal, 2021).

The present study declared that the monocytes and red blood corpuscles were higher in stressed than non-stressed small ruminants. Moreover, the number of monocytes and red blood corpuscles increased significantly in heat stressed goat (Al-Dawood, 2017). However, Heat stress reduces monocytes and circulating red blood corpuscles (Wojtas et al., 2014).

In this study, the values of hematocrit, MCV and MCH were lower in stressed than in the non-stressed small ruminants. However, the values of hematocrit in pigs (Waltz et al., 2014), and MCV and MCH in buffaloes (Haque et al., 2013) increase with stress.

In the current study, the platelet count was higher in the non-stressed than in stressed small ruminants. The mean platelet volume was higher in the infertile stressed than in both fertile non-stressed and stressed animals. Similarly, hyperthermia directly modulates platelet function and can induce cellular damage. Meanwhile, heat stress also affects platelet function via activated coagulation, excess inflammation, production of cytokines (Iba et al., 2023).

In this study, the procalcitonin (PCT) levels were higher in the non-stressed than in the stressed animals. However, systemic infection, major trauma, recent surgery (in human) and mastitis (in Holstein dairy) induce significant PCT rises, which considered as stress biomarker (El-Deeb et al., 2021).

In the present study, the serum glucose concentration was significantly higher in non-stressed than stressed ewes and nanny goats. On the same way, the decrease in glucose level during hyperthermia relates to decrease in concentration of insulin and thyroxine, which are closely associated to energy metabolism during heat exposure (Gupta and Mondal, 2021).

As shown in this study, the serum readings of sodium, potassium, chloride, and magnesium were significantly lower in the non-stressed than in the stressed animals. However, The Na-K ratio was significantly lower in the stressed

than in the non-stressed animals. Similarly, plasma sodium concentration increased in sheep exposed to heat stress (Marai et al., 2008). However, potassium decreased in the sheep plasma and goat serum when exposed to heat stress (Marai et al., 2008). This is occurred due to heat stressed animals lost more potassium in sweat than non-heat stressed animals (Al-Haidary, 2004). On the same direction of the present results, plasma chloride increased significantly with heat stress (Wojtas et al., 2014). This indicates that sheep are able to maintain a normal acid–base balance when heat stress occurred (Srikandakumar et al., 2003). On the contrary of the present results, magnesium decreased in the heat stressed sheep (Čukić et al., 2023). This decrease could be a consequence of possible reduced food intake associated with heat stress (Nazifi et al., 2003).

In the current study, the serum urea level was significantly higher in the non-stressed than in the stressed animals. Besides, the blood ammonia levels were significantly lower in the fertile than in the infertile small ruminants. In agreement of these results, the hot environment reduced nitrogen balance in sheep, probably due to the decrease of total dry matter intake (Dixon et al., 1999). The depression in blood Urea-N associated with exposure of the animals to heat stress may be due to more resorption of the Urea-N from the blood to the rumen to compensate the decrease in ruminal Ammonia-N as a result to the decrease in feed intake (Yousef et al., 1996). Moreover, this decrease in blood Urea-N is due to the increase in urinary nitrogen excretion under severe heat stress conditions as indicated by a negative nitrogen balance (Kamal et al., 1962).

In the present study, the serum creatine phosphokinase concentrations were higher in the infertile than in the fertile animals. On the same bases, human semen samples with low sperm motility and concentrations, high incidence of abnormal sperm morphology, and diminished fertilizing potential have high sperm creatine phosphokinase (CPK) activity (Ünal et al., 2001).

As shown in this study, the serum cardiac troponin (cTnI) levels were significantly higher in the infertile stressed than in the fertile non-stressed animals. On the same direction, the average serum cTnI concentration was lower in the non-stressed than stressed sheep suffered of acute ruminal lactic acidosis (Kirbas et al., 2014). In rats, plasma cardiac troponin I increased 40-fold from the non-stressed to the stressed group (Quinn et al., 2014). cTnI is a clinical biomarker of organ damage and heat stroke in rats (Quinn et al., 2014; Audet et al., 2015) and myocardial injury in neonatal dogs (Pereira et al., 2022). Blood cTnI considered as a biomarker of cardiac diseases and stress (Audet et al., 2015; Tharwat, 2020).

CONCLUSION

In conclusion, heat stress exerted a drastic effect on ewes and nanny goats' reproduction that appears in body temperature, most blood parameters and cardiac troponin I levels. The breeders and veterinarians have to take in their consideration the effect of summer heat stress on ewes and nanny goats' reproduction and try to alleviate this effect during summer months.

AUTHOR CONTRIBUTIONS

Both authors contributed equally.

COMPETING OF INTEREST

The authors declare no competing interests.

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