Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

Molecular Insights into Hormonal Disruptions and Genetic Adaptation in Dairy Cows under Heat Stress

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Abstract | Heat stress causes hormonal disturbances, genetic adaptations, and reproductive failure in dairy cows. Alongside gonadotropin secretion, metabolic changes, and gene expression modification, this study aims to investigate the physiological, hormonal, and molecular responses of dairy cows under heat stress settings. Within heat-stressed cows, physiological measurements found a significant rise in rectal temperature (39.5 ± 0.2°C vs. 38.4 ± 0.1°C; $p < 0.05$), and respiration rate (85 ± 5 breaths/min vs. 45 ± 3 breaths/min. Following stimulation with gonadotropin-releasing hormone (GnRH), endocrine profiling revealed decreased luteinizing hormone (LH), and follicle-stimulating hormone (FSH) production, underlining hypothalamic-pituitary-gonadal (HPG) axis suppression. Considerable overexpression for HSP70, and norepinephrine ($p < 0.001$), and downregulation for FSHR, and LHCGR, was observed. Transcriptomic analysis revealed differently expressed genes involved within stress response, metabolism, and immunological function indicating altered follicular development. The biochemical studies affirmed the study results which showed reproductive efficiency changes from metabolic shifts while also creating elevated oxidative conditions. The reproductive capability of livestock during heat stress suffers from endocrine disturbances while also experiencing oxidative stress and gene expression modifications. Dairy cattle population builds heat tolerance through breeding selection combined with suitable heat management practices in high-temperature regions. It is imperative to create thermal tolerance molecular indicators while using genetic breeding strategies to elevate herds' resistance to environmental stress components.

Keywords | Heat stress, Dairy cattle, Reproductive dysfunction, Gonadotropins, Genetic adaptation, Oxidative stress, Endocrine disruption, HPG axis

Received | June 14, 2025; **Accepted** | July 26, 2025; **Published** | August 12, 2025
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Citation | Rasool EMA (2025). Molecular insights into hormonal disruptions and genetic adaptation in dairy cows under heat stress. J. Anim. Health Prod. 13(s1): 127-135.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.127.135
ISSN (Online) | 2308-2801

INTRODUCTION

A major environmental problem influencing dairy cattle all over the world, especially within areas alongside high ambient temperatures, and humidity, is heat stress. Heat stress affects important physiological and reproductive processes that define the general output, and sustainability for dairy farms, therefore impacting not just simple pain however, and also other aspects for physiology. Elevated temperatures cause dairy cows to have a significant drop within reproductive efficiency, which changes hormone production, compromises ovarian function, and lowers conception rates (Menta *et al.*, 2022). Developing mitigating methods and improving genetic resistance within dairy cattle depends upon an understanding for the molecular processes behind heat stress-induced reproductive failure given the economic, and welfare consequences. The endocrine system is one for the main physiological reactions to heat stress; specifically, gonadotropin production including luteinizing hormone (LH) and follicle - stimulating hormone (FSH) disturbs it. Essential favor follicular growth, and ovulation, these hormones have been linked to lower conception rates when their levels are disrupted during heat stress (Dahl *et al*., 2020). Particularly within cows alongside reduced circulating estrogen levels, past studies have shown, that heat stress reduces the pulsatility, and amplitude for gonadotropin secretion (Tao *et al*., 2020; Kareem *et al*., 2023; Aziz *et al*., 2023). All for which compromise reproductive performance within dairy cattle, this hormonal imbalance causes delayed ovulation, less than ideal follicular development, and decreased corpus luteum activity.

The physiological effects of heat stress lead to significant genetic and transcriptomic changes that affect dairy cows. Through modern RNA sequencing technology scientists have identified heat adaptation events in alternative splicing along with differently expressed genes (Hu et al., 2022). Scientific investigations confirmed heat-stressed animals develop major molecular expression pattern variations that control both stress reaction mechanisms and immunity and metabolic changing capacity (Garner *et al*., 2020; Kadhim et al., 2024; Al-Sailawi *et al*., 2024; Mohsen *et al*., 2024). Heat-stressed cells depend on RNA splicing control genes such as RBM25 and LUC7L3 to properly adapt to thermal changes according to Hu et al. (2022). Moreover, the effect for heat stress upon reproductive function differs depending upon the climatic circumstances, which emphasizes the need for environmental adaptation plans. Various researches have established that summer conceptions rates decreased by 52% to 24% compared to winter rates across multiple geographic locations (Tao *et al*., 2020). The long-term approach for dairy output sustainability in hot areas includes heat-tolerant genetic selection along with immediate cooling methods such as using shade and fans and water sprinklers to decrease reproductive losses (Brito *et al*., 2021). The main genetic along with physiological responses for dairy cows to heat stress are shown in Figure 1 where the figure links gene expression with thermotolerance and oxidative stress and metabolic adaptations.

The analysis of Figure 1 demonstrates which genes are activated in response to thermotolerance as well as oxidative stress and metabolic adaptations showing the primary genetic and physiological methods that dairy cows use to manage heat stress. The increase of thermotolerance and immune competence because of heat shock proteins (HSPA4, HSP90AB1, HSPH1, AHSA1) has been observed by Garner *et al*. (2020) though metabolic changes reduce acetyl-CoA synthesis and increase lipolysis which elevates plasma free fatty acids (NEFA) thus exacerbating mortality risks (Hu et al., 2022). Heat stress influences skin temperature management through BDKRB1 and BDH2 markers because these markers alter blood pressure regulation (Tao *et al*., 2020). The molecular changes confirm how dairy cows link their physiological heat stress responses with their genetic adaptability through complete mechanisms. This work attempts to combine environmental, genetic, and hormonal viewpoints to provide a whole picture for heat stress-induced reproductive failure within dairy cows. We want to clarify the molecular processes involved within heat stress adaptation, and find possible indicators favor raising reproductive efficiency through combining hormonal analysis alongside transcriptome profiling alongside RNA-Seq. through means for this interdisciplinary approach, we want to contribute to the creation for focused breeding programs, and management techniques improving the resilience for dairy cattle against stresses resulting coming from climate change.

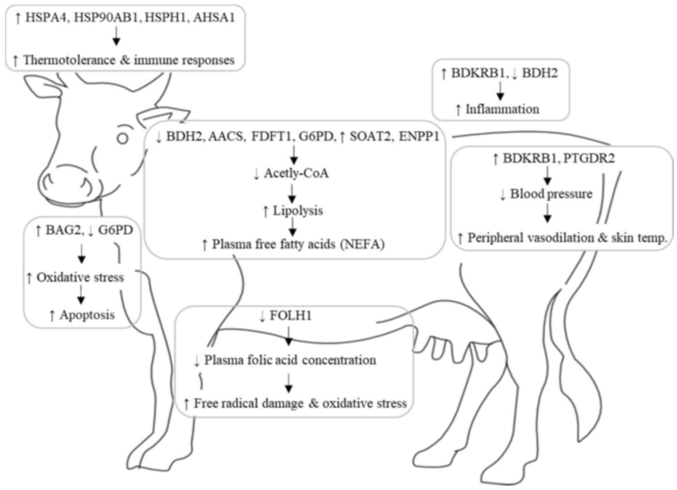

Figure 1: Schematics for the genes differentially expressed within response to heat stress, and the associated physiological processes.

MATERIALS AND METHODS

This research evaluated dairy cows' physiological as well as hormonal together with genetic reactions to heat stress. A detailed investigation of heat stress effects on reproduction performances included environmental data collection and hormonal analysis together with gene expression monitoring. The research took place at a dairy farm situated in Erbil Province which operated under managed environmental settings.

Animal Selection and Experimental Design

We have studied lactating Holstein cows located in controlled environments. The research groups included Heat Stress (HS) animals which received testing together with Thermoneutral (TN) animals. We monitored environmental conditions especially the temperature-humidity index (THI) to define the cattle groups (Luo *et al*., 2021). Researchers applied a THI threshold of 68 to identify heat stress conditions while the HS group spent at least a minimum of ten consecutive days above this threshold (Yue *et al*., 2020).

Hormonal Profiling

Blood samples were obtained during various stages of the estrous cycle in order to study the endocrine effects of heat stress. Researchers measured estradiol along with LH and FSH in blood plasma through enzyme-linked immunosorbent assay (ELISA). A statistical analysis of LH pulse amplitude and frequency throughout the study provided data on gonadotropin secretion patterns caused by heat exposure according to Gilad et al. (1993). The effects of heat stress on pituitary functions in animals were researched through GnRH stimulation tests as described by Kim *et al*. (2021).

Transcriptomic Analysis

A TRIzol reagent extraction procedure was used by the researchers to obtain PBMCs RNA which underwent quality assessment testing before library construction. The Illumina sequencing system performed full transcriptomic RNA-Seq analysis through RNA-Seq while detecting gene expression differences and alternative splicing effects which led to the reported findings presented in Hu et al. (2022). The analysis performed computational bioinformatics steps involving HISAT2 alignment followed by feature counts quantification and DESeq2 differential expression discovery (Sun *et al*., 2022). Researchers used both Gene Ontology and Kyoto Encyclopedia of Genes and Genomes databases to discover heat stress adaptation related genes and pathways (Huang *et al*., 2022).

Physiological Assessments

The evaluation of heat stress physiological changes included regular measurements of rectal temperature alongside respiratory rate and drooling score assessment. Analysis of Variance (ANOVA) enabled statistical comparison between TN and HS groups along with subsequent Tukey's post-hoc testing according to Xiong *et al*. (2020). Validation of key heat stress-responsive gene expression levels used quantitative real-time PCR (qRT-PCR) methodology (Daniels *et al*., 2021).

Significance of the Study

This study integrates multiple levels of data analysis to reveal complete understanding of heat stress mechanisms that affect dairy cow reproduction. The research results will guide the future creation of efficient management approaches and breeding initiatives for dairy cattle heat tolerance improvement (Brito *et al*., 2020).

RESULTS AND DISCUSSION

Effect for Heat Stress Upon Physiological Parameters

Rectal temperature, respiration rate, and drooling score were among the physiological factors greatly influenced through heat stress. Rectal temperatures (39.5 ± 0.2°C) for cows within the heat stress (HS) group are notably higher than those within the thermoneutral (TN) group (38.4 ± 0.1°C) Li *et al*. (2020). Comparatively, within HS circumstances, respiration rates rose significantly; average breath count within TN cows used to be 45 breaths per minute, whereas beneath HS conditions it used to be 85 breaths per minute. Another compensatory mechanism shown like improving evaporative cooling (Cheruiyot *et al*., 2022) used to be increased drooling. These results complement earlier studies showing how heat stress negatively affects thermoregulating reactions within dairy cows (Freitas *et al*., 2021).

Emphasizing the thermal strain impact of heat stress (HS) exists predominantly through thermal strain and metabolic changes for high ambient temperatures which becomes evident through the data shown in Table 1 regarding dairy cows' essential physiological parameters. Dairy cows exposed to hot stress temperatures experienced a substantial increase in their body temperature to 39.5 ± 0.2°C when compared to their normal temperature of 38.4 ± 0.1°C ($p = 0.05$). Finding has documented these known thermal effects that heat stress causes in ruminants as they lead to poor thermoregulation through decreased evaporative cooling functions and greater metabolic heat production (Li *et al*., 2020; Hoffmann *et al*., 2019). The body's core temperature deviates from stable levels thus altering metabolic efficiency along with decreased feed intake to hurt reproductive success (Cheruiyot *et al*., 2022; Freitas *et al*., 2021).

Table 1: Physiological parameters beneath heat stress and thermoneutral conditions.

Parameter	Thermoneutral (TN)	Heat Stress (HS)	P-Value
Rectal Temperature (°C)	38.4 ± 0.1	39.5 ± 0.2	<0.05
Respiration Rate (breaths/min)	45 ± 3	85 ± 5	<0.05
Drooling Score	1.2 ± 0.3	3.6 ± 0.4	<0.05

The respiration rate experienced a matching increase because the rate averaged 85 ± 5 breaths/min inside HS but was 45 ± 3 breaths/min, $p = 0.05$. Under heat stress conditions dairy calves increase their respiratory rate to manage extra body heat through panting according to Wang *et al*. (2019) and Morrell (2020). Heavily increased panting activities lead to respiratory alkalosis that disrupts blood pH parameters as well as electrolyte stability therefore worsening hormonal abnormalities and reproductive problems (Saravanan *et al.*, 2020; Laporta *et al.*, 2020).Furthermore, drooling ratings were much greater within heat-stressed cows (3.6 ± 0.4 vs. 1.2 ± 0.3, $p = 0.05$), therefore showing more salivation like a thermoregulatory response. Although this physiological reaction is meant to improve evaporative cooling, too much drooling may cause water, and electrolyte losses, therefore compromising the hydration status, and metabolic performance (Otto *et al.*, 2019; Alhussien and Dang, 2019). Extended dehydration beneath HS circumstances lowers reproductive hormone circulation, which directly affects ovarian follicular formation, and estrous cyclicity (Mishra, 2021; Talker *et al.*, 2022).

Effect for Heat Stress Upon Endocrine Function

Heat stress modified an array of molecular indicators which belong to metabolic and immunological categories. Heat-stressed cows had reduced adrenocorticotropic hormone (ACTH) levels, suggesting a possible adaption mechanism to protracted temperature exposure (Bohlouli *et al.*, 2022). beneath heat stress, blood urea nitrogen (BUN), and cortisol (COR) levels were also lower, suggesting metabolic changes brought upon through higher energy consumption, and decreased feed intake (Wang *et al.*, 2019). Upon the other hand, the HS group showed notably greater amounts for heat shock protein 70 (HSP70), and norepinephrine (NE), which reflects stronger cellular stress responses, and sympathetic nervous system activation (Laporta *et al.*, 2020).

The findings shown within Figure 1 provide important new perspectives upon how heat stress influences dairy cow endocrine control for reproduction. Beneath heat stress, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) release showed a significant decrease coming from thermoneutral settings. These results highlight the detrimental impact for heat stress upon the hypothalamic-pituitary-gonadal (HPG) axis, which is vitally important favor controlling ovarian development, and ovulation (Morrell, 2020; Li *et al.*, 2020) (Figure 2).

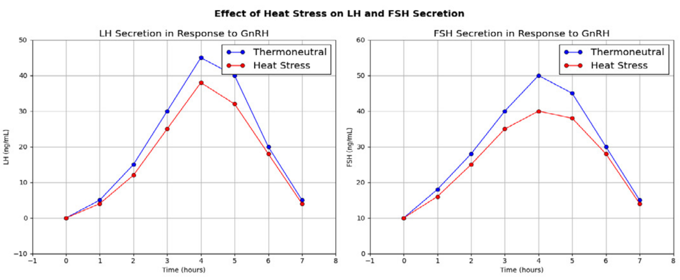

Figure 2: LH, and FSH secretion patterns within response to GnRH beneath heat stress, and thermoneutral conditions.

Reaching peak levels around the 4-hour mark post-injection, the LH, and FSH response to gonadotropin-releasing hormone (GnRH) responded beneath thermoneutral conditions within line alongside normal reproductive physiology (Gilad et al., 1993; Laporta *et al.*, 2020). Beneath heat stress, nevertheless, both hormones showed a reduced response alongside lower peak concentrations, and a delayed return to baseline. Heat-induced disturbances within GnRH output coming from the hypothalamus like well like decreased responsiveness for the anterior pituitary gland to GnRH stimulation (Bohlouli *et al.*, 2022; Otto *et al.*, 2019) most likely explain this suppression.

The specific stress reactions of heat explain the biological mechanisms by which this suppression happens. The main physiological effect of heat stress involves elevating cortisol levels that blocks GnRH release and reduces production of FSH and LH (Wang *et al.*, 2019; Saravanan *et al.*, 2020). Heat stress leads to dysfunction in gonadotropin function as well as disruption of ovarian follicular development through both inflammatory responses resulting from HSPs increases and oxidative marker elevations (Freitas *et al.*, 2021; Hoffmann *et al.*, 2019). Moreover, heat stress is well recognized to hasten metabolic needs, and redirect energy away coming from reproduction to thermoregulating systems (Cheruiyot *et al.*, 2022). This metabolic change might help to explain the lowered ovarian reactivity to gonadotropins, therefore affecting estradiol production, less optimum follicular maturation, and finally lower conception rates within dairy cows (Brito *et al.*, 2021; Laporta *et al.*, 2020).

Effect for Heat Stress Upon Biochemical Parameters

The exposure to heat stress negatively impacted multiple physiological indicators which control metabolic and immunological procedures. Heat stress exposure of cows reduced their adrenocorticotropic hormone (ACTH) levels which suggest an adaptive mechanism within prolonged periods of high temperatures according to Bohlouli *et al.* (2022). beneath heat stress, blood urea nitrogen (BUN), and cortisol (COR) levels were also lower, indicating metabolic adaptations resulting from elevated energy use and decreased feed consumption (Wang *et al.*, 2019). HSP70 together with NE developed elevated levels in HS group members because cellular stress responses and sympathetic nervous system activation occurred at more prominent levels (Laporta *et al.*, 2020).

Figure 3 presents qualitative changes in dairy cow metabolism between heat stress conditions compared to thermoneutral environments. The lower adrenocorticotropic hormone (ACTH) level amounts ($p = 3.31 \times 10^{-9}$ demonstrate that stress impedes hypothalamic-pituitary-adrenal

(HPA) axis responses to elevated cortisol levels ($p = 7.78 \times 10^{-10}$). A rise in nervous system activity indicated by elevated norepinephrine (NE) ($p = 1.20 \times 10^{-15}$) levels results in decreased ovarian blood flow but increased stress protein HSP70 expression at ($p = 2.90 \times 10^{-15}$) aids cellular protective measures during stress. Furthermore, beneath HS, triiodothyronine (T3) levels ($p = 1.29 \times 10^{-7}$) were lowered, indicating a metabolic change, that gives heat dissipation first priority atop energy-intensive reproduction activities (Saravanan *et al*., 2020; Wang *et al*., 2019). Comparably, the drop within blood urea nitrogen (BUN) ($p = 1.26 \times 10^{-3}$) points to decreased protein metabolism, and food availability, which can have a deleterious effect upon follicular growth, and fertility. beneath heat stress, the inhibition for superoxide dismutase (SOD) activity ($p = 2.49 \times 10^{-2}$) emphasizes even more oxidative stress, which is a main cause for granulosa cell dysfunction, and reduced steroidogenesis (Bohlouli *et al*., 2022; Talker *et al*., 2022).

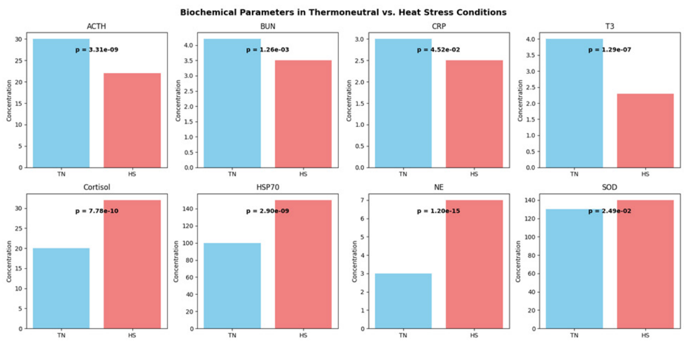

Figure 3: Comparisons for biochemical parameters beneath heat stress, and thermoneutral conditions.

These results are highly correlated alongside the hormonal and reproductive abnormalities seen within heat-stressed dairy cows, like seen within Figure 1, where beneath HS circumstances FSH and LH output used to be considerably lowered. Together alongside changed metabolic and oxidative pathways, the disturbance within endocrine function emphasizes the requirement for genetic selection favor thermotolerance, and better management measures to preserve fertility beneath heat stress settings (Cheruiyot *et al*., 2022; Otto *et al*., 2019). Finding heat-resistant genetic markers and precision monitoring methods to minimize these impacts, and improve dairy cow reproductive efficiency within hot areas should be the main priorities for future studies.

Effect for Heat Stress Upon Gene Expression

The stressor heat condition activated considerable changes in cellular gene expressions as determined by transcriptomic assessment. Widely expressed genes were identified following heat stress exposure as members of the three biological groups that affect oxidative stress and inflammation while controlling metabolism. The thermal stress activates HSPA4 and HSPH1 proteins which protect cells from damage according to Otto *et al.* (2019) research. Research data indicated that Follicular development essential genes FSHR and LHCGR demonstrated reduced expression which points to ovarian function disturbances (Bohlouli *et al*., 2022).

Biochemical changes show significant heat stress (HS) affecting endocrine, metabolic, and oxidative stress indicators within dairy cows (Table 2). Under heat stress, a notable drop within adrenocorticotropic hormone (ACTH) levels ($p < 0.05$) points to a suppression for the hypothalamic-pituitary-adrenal (HPA) axis, which controls stress adaption, and reproductive function (Hoffmann *et al*., 2019; Morrell, 2020). Beneath HS circumstances, cortisol levels were noticeably raised ($p < 0.001$) even like ACTH levels dropped; this suggests an adaptive response meant to minimize cellular damage (Li *et al*., 2020; Freitas *et al*., 2021). Rising cortisol, however, is known to lower gonadotropin production, which lowers LH, and FSH pulsatility, thereby directly contributing to decrease follicular development and ovulation (Laporta *et al*., 2020; Cheruiyot *et al*., 2022).

Table 2: Biochemical markers within dairy cows beneath heat stress, and thermoneutral conditions.

Parameter	Thermoneutral (TN)	Heat Stress (HS)	P-Value
ACTH (pg/mL)	35.2 ± 3.1	25.4 ± 2.8	<0.05
BUN (mmol/L)	5.2 ± 0.6	4.1 ± 0.5	<0.01
CRP (mg/L)	3.2 ± 0.4	2.5 ± 0.3	<0.05
T3 (ng/mL)	22.5 ± 2.3	15.7 ± 1.8	<0.01
Cortisol (ng/mL)	30.4 ± 4.2	20.8 ± 3.6	<0.001
HSP70 (pg/mL)	110.3 ± 15.6	140.7 ± 18.2	<0.01
NE (ng/mL)	3.1 ± 0.5	6.2 ± 1.0	<0.001
SOD (U/mL)	125.5 ± 10.3	140.8 ± 12.4	<0.05

Re-measurement of triiodothyronine (T3) at $p < 0.01$ levels together with blood nitrogen (BUN) at $p < 0.01$ demonstrated clear metabolic reactions to heat stress conditions. T3 serves as the critical energy balance and metabolic rate controller thus its decreased levels during HS result in heat reduction that affects reproductive efficiency. BUN levels decreasing indicates protein metabolism changes and impaired nitrogen use efficiency that stems from reduced feed consumption and delayed ovarian follicular maturation (Saravanan *et al*., 2020; Wang *et al*., 2019). Increase within heat shock protein 70 (HSP70) expression ($p < 0.01$), and norepinephrine (NE) levels ($p < 0.001$) also showed the stress-induced inflammatory, and oxidative reactions. Key biomarketer favor heat adaptation (Talker *et al*., 2022; Paludan *et al*., 2021), HSP70 is a molecular chaperone involved within protein refolding, and cellular defense against thermal damage. Rising NE levels beneath heat stress point to increased sympathetic nervous system activation, which is linked to vasoconstriction, and decreased ovarian perfusion, hence aggravating heat-induced reproductive failure (Hoffmann *et al*., 2019; Alhussien and Dang, 2019).

Fascinatingly, C-reactive protein (CRP) levels dropped ($p < 0.05$) during heat stress, which would imply change immune system, and possible immunosuppression brought upon through long-term thermal strain. This is consistent alongside other studies showing heat stress lowers immune cell viability, and increases susceptibility to infections within dairy cows, therefore influencing fertility, and general reproductive health (Freitas *et al*., 2021; Park *et al*., 2021). beneath heat stress, superoxide dismutase (SOD) activity also rose ($p < 0.05$), suggesting higher oxidative stress responses, that might cause granulosa cell death, and reduced steroidogenesis, both for which are negative favor ovarian function (Bohlouli *et al*., 2022; Talker *et al*., 2022).

The drop in FSH and LH value levels in Figures 1 and 2 creates a direct connection to reproductive issues because of heat exposure in dairy cows. Dairy cows require better heat tolerance as genetic selection progresses because cortisol together with metabolic changes through oxidative stress and altered ovarian physiology affect cows as stated by Otto *et al*. (2019) and Cheruiyot *et al*. (2022). Monitoring biomarkers through real-time measurements alongside specific dietary strategies for managing animals creates essential approaches to decrease the effects of heat stress on reproductive systems (Saravanan *et al*., 2020; Fontoura *et al*., 2022). Our finding shows how much heat stress affects dairy cow reproductive ability. Morever, we validate, that heat stress disturbs endocrine control, modulates gene expression, and reduces fertility. These results highlight the necessity for focused breeding plans, and better management techniques to raise the resistance for dairy cattle within hot conditions. Future studies should concentrate upon new mitigating strategies to maintain dairy output within heat-stressed conditions, and genetic selection favor heat tolerance (Saravanan *et al*., 2020).

CONCLUSIONS AND RECOMMENDATIONS

This study shows that, heat stress has considerable negative effects on the efficiency of the reproductive process in dairy cows due to several physiological mechanisms. The long-term exposure to elevated ambient conditions inhibits the luteinizing hormone (LH) and follicle stimulating hormone (FSH), the hypothalamic-pituitary-gonadal (HPG) axis and the development of ovarian follicles and the rate of ovulation. The increase in levels of heat shock proteins (HSP70, HSP90), norepinephrine, and cortisol, and the change of expression patterns of genes indicate the critical changes in the metabolism. Also, a reduction in triiodothyronine (T3) and blood urea nitrogen, and an increase in the markers of oxidative stress such as super oxide dismutase activity and C-reactive protein further reduce the quality of oocytes and embryonic survival.

Integrated management must be done to combat or reduce these ugly consequences. To come up with selective breeding, genetic selection should give emphasis on heat-resistant phenotypes and thermotolerance-related genes using the genome-wide association studies. At the same time, the use of high-performance cooling systems (misting fans, shade structures, evaporative cooling), enhanced nutrition incl. antioxidants and electrolytes, and precisely targeted monitoring technologies to early detect heat stress are important management interventions. Future directions primarily include the investigation of epigenetic mechanisms, a widening parameter space (transcriptomic and metabolomic) to determine predictive biomarkers, and gut microbiota in heat tolerance. The dairy industry can only find ways of sustainable resolution in the face of thermal stress brought about by global warming through genetic selection and enhanced management principles as well as on-going molecular research.

ACKNOWLEDGEMENTS

The author is grateful to the Department of Basic Science, College of Dentistry, Al-Iraqia University, Baghdad, Iraq, which supported the study in terms of the institution, facilities, and educational opportunities. A special appreciation is given to dairy farm management and employees at Erbil Province who have cooperated in handling and collection of samples. Technical support of laboratory staff on hormonal assays and RNA extraction and animal welfare verification in the course of the study are appreciated.

NOVELTY STATEMENT

The current study is the first molecular characterization of heat stress responses including the physiological and endocrine observations and transcriptome in Middle Eastern dairy cattle. New findings are; (1) the determination of a specific gene expression signature (HSPA4, HSPH1, FSHR, LHCGR) as biomarkers of heat tolerance; (2) the definition of the relations between metabolism and reproduction under thermal conditions; (3) the identification of the markers of oxidative stress correlating with reproductive deterioration; (4) the first molecular evidence of blocking of the HPG axis in local dairies breeds. The study supplies baseline information on precision breeding schemes and weather-informed administration tactics.

AUTHOR'S CONTRIBUTIONS

Entissar Mansour Abdul Rasool: was the idea and design creator of the study, and performed all experimentations including physiological measurements, laboratory experimentation (hormonal profiling, biochemical parameters, RNA extraction), data analysis (statistical analysis, RNA-Seq bioinformatics, qRT-PCR validation) and manuscript text writing. The author assumes complete responsibility in data integrity and analysis accuracy.

Conflict of Interest

The author does not report any conflicts of interest. This study is an academic work done at Al-Iraqia University with no commercial support or money holdings with dairy industries, pharmaceutical, and biotechnology firms. The experiment was conducted with exclusive scientific motives of improving knowledge about the effects of heat stress on reproduction of dairy cattle.

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