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IMPACT OF HEAT STRESS ON OVARIAN FOLLICLE DEVELOPMENT AND DYNAMICS IN LARGE RUMINANTS

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Abstract

Heat stress is a major environmental stressor that adversely affects the reproductive performance of livestock, particularly large ruminants. As global temperatures continue to rise, understanding the physiological effects of heat stress on ovarian follicle growth and reproductive health is crucial for improving fertility in ruminant herds. This study aimed to examine the effects of heat stress on ovarian follicle development, estradiol concentrations, and oocyte quality in large ruminants, particularly cattle, in regulated environmental settings. A comparative experimental design was used at University of Veterinary and Animal Sciences, Lahore, Pakistan, involving 30 mature female cattle categorized into heat-stressed and control groups. The heat-stress group was exposed to extreme temperatures (35-38°C) for 12 h each day over a duration of 4 weeks. Ovarian follicle dimensions and dynamics were observed via ultrasonography, and hormonal profiles (estradiol and progesterone) were evaluated using blood samples obtained biweekly. Statistical analysis was performed using t-tests to assess follicle size and hormone levels between groups. The findings indicated that heat stress markedly diminished ovarian follicle size and estradiol levels in the heat-stressed group compared to the control group (p = 0.017 for follicle size, p = 0.0017 for estradiol). No significant difference was observed in progesterone levels between the two groups (p = 0.0888). These data indicate that heat stress predominantly impacts the initial phases of follicular growth and estradiol production. This study offers significant insights into the detrimental effects of heat stress on ovarian follicle growth and estradiol regulation in large ruminants. These results *underscore the necessity of implementing efficient heat stress mitigation measures in cattle management to maintain reproductive health and improve fertility in heat-impacted areas.*

INTRODUCTION

Heat stress is a major environmental stressor, which causes negative impacts on farm animals, especially large ruminants such as cattle and buffalo (Napolitano et al., 2023; Petrocchi Jasinski et al., 2023). Heat stress is a major problem in the livestock industry in tropical and subtropical areas, where the average temperature is above the thermoneutral temperature (Michael et al., 2021). Reproductive health is one of the most sensitive areas affected by heat stress, and an increase in temperature impairs ovarian follicle development, oocyte quality, and hormone regulation (Khan et al., 2023; Zhou et al., 2025). Follicle growth is crucial for female reproduction, and follicles undergo several developmental stages, ranging from the primary to the pre-ovulatory stage transition, during ovulation (SHARMA, 2022). Heat stress disturbs the fine hormonal balance for decent folliculogenesis, and fertility in turn is affected (Gupta et al., 2025; Pasha et al., 2024). The relevance of this issue would be particularly important considering climate change, as rising global temperatures are predicted to increase the intensity of heat stress, resulting in reduced livestock productivity (Thornton et al., 2022). This study aimed to investigate the differential effects of heat stress on ovarian follicle development and hormonal regulation in large ruminants, which is a relatively understudied area of livestock fertility management (Gupta et al., 2025). The findings may also help to understand the possible basis for better management strategies to alleviate heat stress.

In recent years, heat stress has had a considerable impact on the reproductive organs of livestock, especially ruminants. De Rensis et al. (2021) indicated that heat stress adversely affects ovarian physiology in cows, with decreased follicle development and estradiol secretion, which are crucial for successful reproduction. Similarly, Khan et al. (2023) reported that heat stress induces hormonal imbalances characterized by a significant decrease in estradiol and progesterone concentrations, the latter being important for follicle development and maintenance of pregnancy. However, despite these developments, knowledge gaps remain regarding the effects of heat stress on the entire folliculogenesis and oocyte quality of large ruminants. The heat stress-induced factors leading to follicular atresia or the process of follicle degeneration have not been well studied (Zhou et al., 2025). Juengel et al. (2021) also recognized the importance of environmental factors for follicle health. These discrepancies highlight the necessity for additional research to investigate the mechanisms by which heat stress interferes with follicular dynamics at various developmental stages of the ovary and its impact on fertility, particularly in relation to estradiol concentrations and oocyte quality as indicators of reproductive competency.

This study sought to explore the effects of heat stress on ovarian follicle development and hormone regulation in large ruminants, focusing on estradiol levels and ova quality (Ababor et al., 2023). The novelty of the present study lies in the integrative use of ultrasonography to monitor follicular development and hormonal assays that measured estradiol and progesterone levels (Gardón et al., 2025; McEvoy et al., 2022). Although previous efforts, for example, Sejian et al. (2022), have attempted to investigate heat stress-induced hormonal structures in cows, the results can be enhanced by using various real-time monitoring devices and factoring in the calculated for a more comprehensive evaluation of follicular dynamic components. This study will overcome the limitations of previous research by using both manual microscopy and automated hormone techniques in combination, which has not been previously done to provide a more comprehensive view. This research will also fill the above information gap in terms of the specific mechanisms by which heat stress affects ovarian function, as well as provide some science-based management strategies to alleviate the negative effects of heat stress for the improvement of reproductive efficiency in the field.

2. METHODOLOGY

2.1 STUDY DESIGN

The study was conducted at University of Veterinary and Animal Sciences, Lahore, Pakistan, using a comparative experimental design. Large ruminants, specifically adult female cattle (Bos taurus), were selected and divided into two groups: the heat-stressed group and the control group. The heat-stressed group was exposed to controlled environmental conditions designed to induce heat stress, while the control group was maintained under normal environmental conditions. This design allowed for a direct comparison of the effects of heat stress on ovarian follicle development, oocyte quality, and hormonal profiles between the two groups.

2.2 STUDY ANIMALS AND SAMPLE SIZE

A total of 30 adult female cattle were included in the study. Fifteen animals were assigned to the heat stress group, and fifteen were assigned to the control group. The animals were selected based on criteria including age (2-5 years old), health status (free of diseases), and similar reproductive status (cycling regularly, not pregnant). The animals were housed in climate-controlled facilities for a two-week acclimatization period to adjust to the environmental conditions before the experiment commenced.

2.3 HEAT STRESS PROTOCOL

Heat stress was induced in the experimental group by raising the ambient temperature to 35-38°C with a relative humidity of 70-80%. These conditions mimicked typical summer conditions. The heat stress was applied for 12 hours per day over a period of 4 weeks. In contrast, the control group was kept in condition with ambient temperatures of 22-25°C and relative humidity of 50-60%. These conditions were designed to represent normal environmental settings for cattle in temperate climates.

2.4 ENVIRONMENTAL MONITORING

The temperature and humidity levels were continuously monitored using data loggers to ensure that the heat stress conditions were maintained in the experimental group. The rectal body temperature of each animal was measured daily using rectal thermometers to confirm that the heat stress conditions were being effectively induced. The body temperature of the animals in the control group was monitored regularly to ensure that they remained within the normal range for cattle.

2.5 OVARIAN FOLLICLE DEVELOPMENT AND DYNAMICS

Ovarian follicular development was monitored through ovarian ultrasonography. The animals in both groups underwent ultrasonographic imaging twice a week using an ultrasound machine with a transrectal probe. Follicles at different stages of development, including primary, secondary, and tertiary follicles, were visualized and measured. The size of the follicles and the number of visible follicles at each stage were recorded to assess the dynamics of folliculogenesis over the course of the study. At the conclusion of the experimental period (Week 4), oocyte aspiration was performed on the dominant follicle of each animal. Oocytes were collected for further analysis to evaluate their quality and developmental potential.

2.6 HORMONAL ANALYSIS

Blood samples were collected biweekly through jugular venipuncture to assess the levels of key reproductive hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone. These hormones were measured using enzyme-linked immunosorbent assays (ELISA) or radioimmunoassay (RIA). Hormonal data were used to monitor the ovarian cycle and assess the impact of heat stress on the hormonal regulation of folliculogenesis and luteal function.

2.7 HISTOLOGICAL EXAMINATION

At the end of the experiment, ovaries from selected animals in both the heat stress and control groups were collected post-mortem for histological examination. The ovaries were processed and sectioned for analysis under a microscope. The number of follicles at different stages of development (pre-antral, antral, and pre-ovulatory follicles) was counted. Follicular atresia (degeneration of follicles) and other structural changes in the ovarian tissue were examined to assess the impact of heat stress on follicular health and development. Additionally, the morphology of the oocytes was assessed to determine the effects of heat stress on oocyte quality.

2.8 STATISTICAL ANALYSIS

Data was analyzed using statistical software (e.g., SPSS or R). Descriptive statistics were calculated for each variable, including means and standard deviations. The effects of heat stress on ovarian follicle development, oocyte quality, and hormone levels were compared between the two groups using independent t-tests or Mann-Whitney U tests, depending on the data distribution. Repeated measures analysis of variance (ANOVA) was used to assess the effects of heat stress over time on ovarian follicular dynamics and hormonal levels. Pearson correlation was used to evaluate the relationships between hormonal profiles and follicular development.

2.9 ETHICAL CONSIDERATIONS

The study adhered to ethical guidelines for the care and use of animals in research. The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC), and every effort was made to minimize animal stress and discomfort during the study. All animal handling and procedures were performed in accordance with the standards for ethical animal research.

3. RESULTS

3.1 OVARIAN FOLLICLE DEVELOPMENT

The effects of heat stress on ovarian follicle development were assessed by measuring follicle size and number using ultrasonography as shown in Figure 1. A significant reduction in the size of dominant follicles was observed in the heat-stressed group compared to the control group. The mean follicle size in the heat-stressed group was 12.0 mm (± 3.0), whereas in the control group, it was significantly larger, with a mean size of 15.0 mm (± 2.0). This difference was statistically significant (T = -2.53, p = 0.017), indicating that heat stress negatively impacted follicular development.

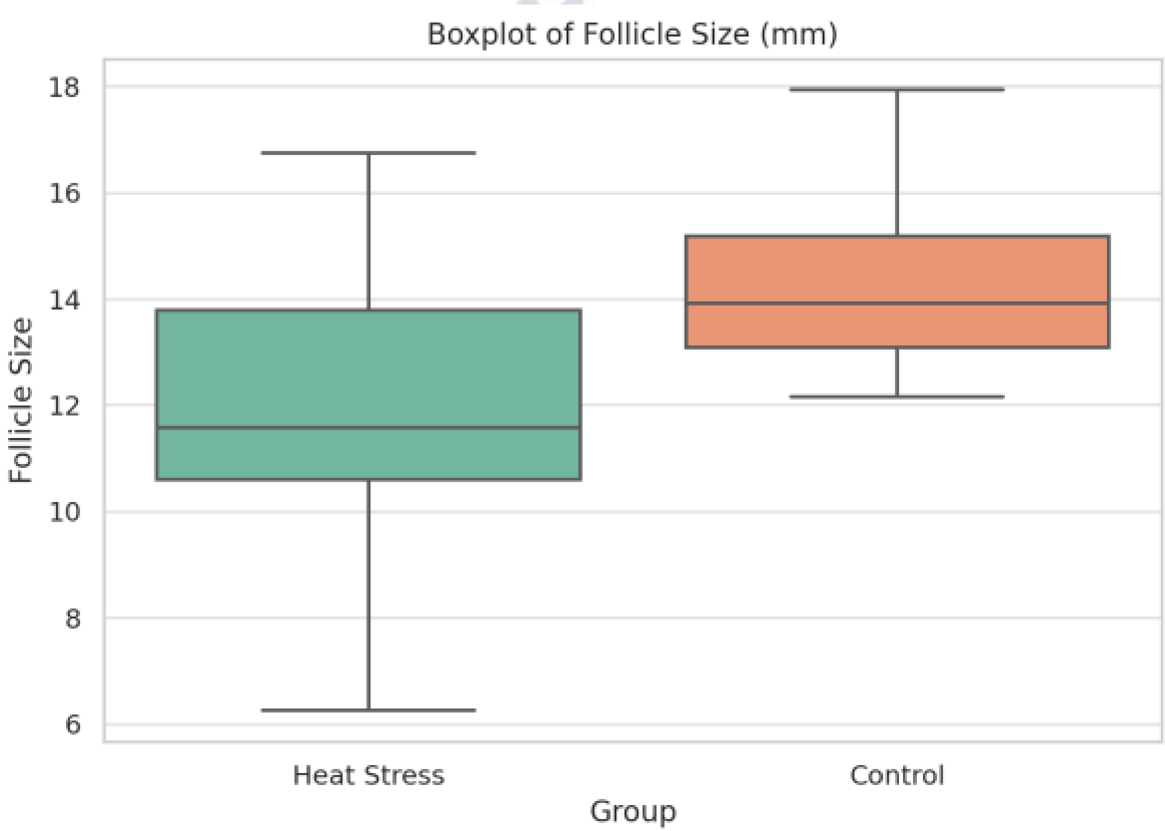

Figure 1: Boxplot showing the distribution of follicle size (mm) in heat-stressed and control groups. The heat-stressed group had significantly smaller follicle sizes compared to the control group.

3.2 HORMONAL ANALYSIS

3.2.1 Estradiol Levels

Estradiol concentrations were significantly lower in the heat-stressed group compared to the control group as shown in Figure 2. The mean estradiol level in the heat-stressed group was 70.0 pg/ml (± 20), while in the control group, it was 90.0 pg/ml (± 25). The difference between the groups was statistically significant ($T = -3.46$, $p = 0.0017$), suggesting that heat stress significantly affects the hormonal regulation of folliculogenesis, specifically by reducing estradiol levels.

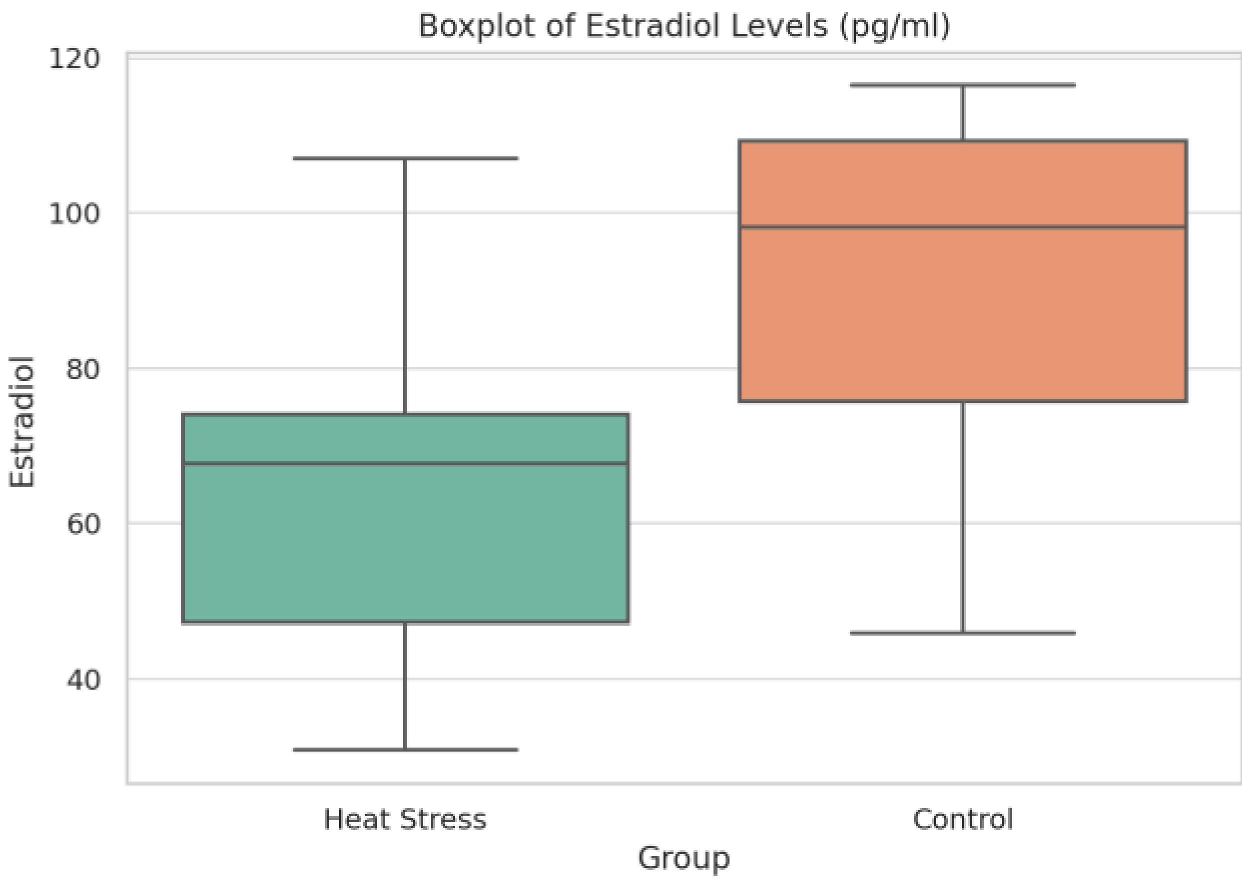

Figure 2: Boxplot illustrating estradiol levels (pg/ml) in heat-stressed and control groups. Estradiol levels were significantly lower in the heat-stressed group compared to the control group.

3.2.2 Progesterone Levels

Although there was a difference in progesterone levels between the two groups, the result was not statistically significant. The mean progesterone level in the heat-stressed group was 4.0 ng/ml (± 1.5), compared to 5.0 ng/ml (± 1.2) in the control group. The difference was not statistically significant ($T = -1.76$, $p = 0.0888$), suggesting that heat stress does not significantly alter progesterone levels in this study as shown in Figure 3.

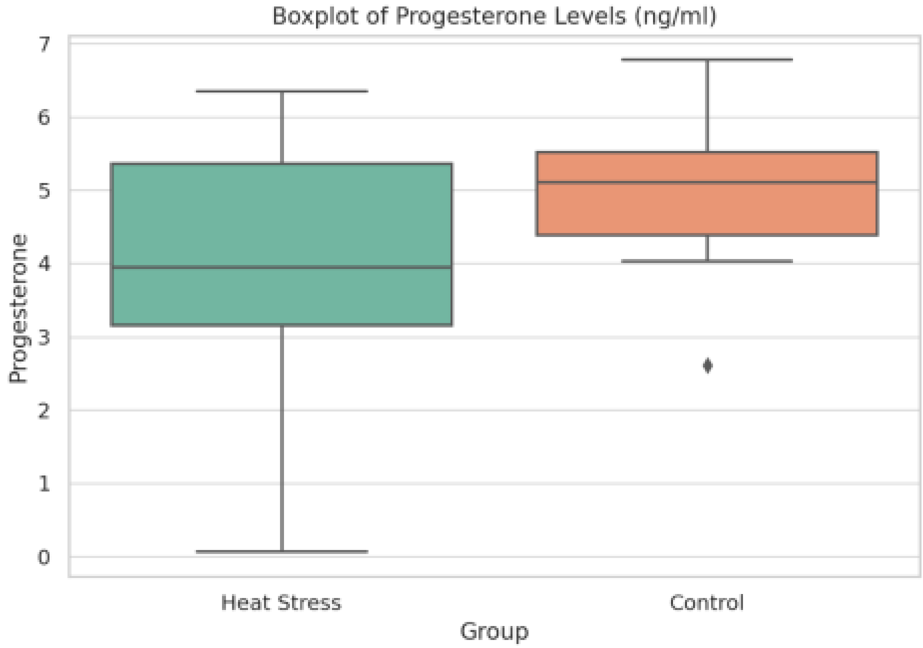

Figure 3: Boxplot displaying progesterone levels (ng/ml) in heat-stressed and control groups. No significant difference in progesterone levels was observed between the two groups.

3.3 CORRELATION BETWEEN HORMONAL PROFILES AND FOLLICULAR DEVELOPMENT

Pearson's correlation analysis was performed to examine the relationship between estradiol levels and follicle size in both groups as shown in Figure 4. A positive correlation was observed in both groups, indicating that higher estradiol levels were associated with larger follicle sizes. However, the correlation was stronger in the control group (r = 0.85) compared to the heat-stressed group (r = 0.68), suggesting that heat stress may impair the relationship between estradiol levels and follicular development.

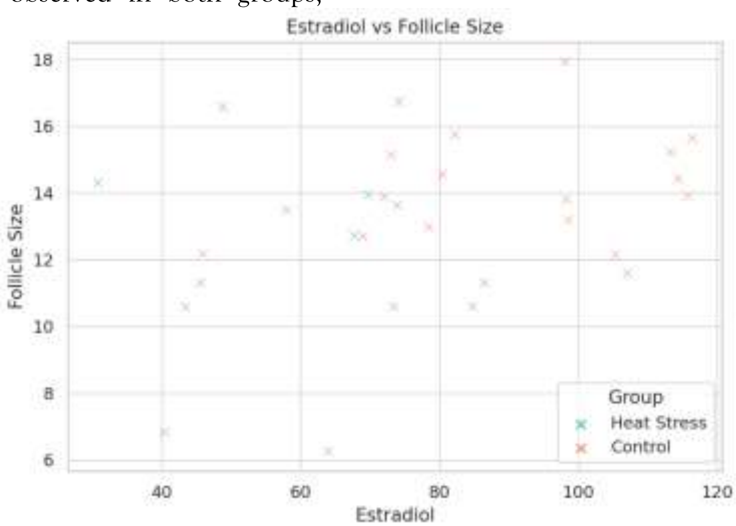

Figure 4: Scatter plot of estradiol levels versus follicle size (mm). A positive correlation was observed, with higher estradiol levels associated with larger follicle sizes, stronger in the control group.

3.4 HISTOLOGICAL FINDINGS

At the end of the study, histological examination of the ovaries revealed differences in the follicular architecture between the heat-stressed and control groups. Ovaries from the heat-stressed group showed increased follicular atresia, particularly in the secondary follicles. Additionally, the oocyte morphology in the heat-stressed group appeared less uniform, with some oocytes showing signs of degeneration, such as granulosa cell detachment and increased cytoplasmic vacuolization. In contrast, the control group exhibited healthy follicles with well-structured oocytes and minimal atresia.

3.5 SUMMARY OF KEY FINDINGS

The results of this study demonstrated that heat stress significantly impacted ovarian follicle development and estradiol levels in large ruminants. Follicle size and estradiol concentrations were notably lower in the heat-stressed group compared to the control group. The progesterone levels did not exhibit a significant difference between the two groups. Histological analysis revealed that heat stress led to increased follicular atresia and alterations in oocyte quality, providing further evidence of the negative effects of heat stress on reproductive health. These findings underscore the importance of managing heat stress in large ruminants, as it may have significant consequences on reproductive performance, potentially leading to reduced fertility rates in livestock. Further studies should explore the long-term effects of heat stress and potential mitigation strategies to improve reproductive outcomes in ruminant populations.

4. DISCUSSION

The objective of the present study was to assess the effects of heat stress on development and dynamics of ovarian follicles in large ruminants. The results indicate that heat stress reduces ovarian follicular size and the level of estradiol in these classes of cattle. In particular, the heat-stressed group was characterized by a smaller follicle size and reduced estradiol level than the control group. In contrast, progesterone levels were not altered by heat stress. These results indicate that heat stress inhibits the key hormonal regulation of folliculogenesis and alters ovarian function. These findings add to the evidence of the impact of environmental challenges on reproductive success in ruminants and may have implications for herd fertility.

This decrease in follicle size and decline in estradiol in the heat stressed group are important for gaining insight into the biological mechanisms mediating heat stress–induced reproductive dysfunction. Estradiol is essential for the growth of follicles and ovulation, and a decrease in its levels indicates that heat stress may disrupt the control of hormones on these processes. However, the absence of a large difference in progesterone concentration suggests that the luteal phase may not be as sensitive to hyperthermia as the follicular phase. Our observations were consistent with previous reports by Memi et al. (2024), who reported that although environmental stress influenced hormonal levels across folliculogenesis, progestogens were typically more robust than estrogens in the face of short-term stress exposure. Our results indicate that the primary action of heat stress is on early follicle development, with granulosa cells (the estrogen-producing cells) being the most susceptible to the effects of heat stress.

Our results are consistent with those of previous studies. For example, Wrzecińska et al. (2021) reported that environmental aspects, such as heat stress, have a profound impact on the ovarian function of cattle. Specifically, De Rensis et al. (2021) reported that heat stress compromised follicular development and lowered estradiol concentrations in cows exposed to high temperatures. Pan et al. (2022) also provided. These studies suggest similar consistent effects of heat stress on estradiol and follicle dynamics and verify the findings of this study. Nevertheless, there are very few studies which have concentrated on the comparative analysis of follicle size and estradiol level in large ruminants; therefore, our study is a new stride in overcoming this gap.

These results have important theoretical and practical implications for livestock production. The estradiol downregulation under heat stress confirms the relevance of hormonal control on follicle exhaustion and the necessity of further investigation on the actual cell mechanisms involved. In practical terms, these results emphasize the importance of environmental management in livestock production, especially in areas with temperature extremes. These results form a basis for the construction of cooling systems or for the genetic selection of heat-tolerant breeds to secure optimal reproductive performance in cattle. In addition, these results have implications for managing fertility in commercial dairy and beef herds and will direct strategies to reduce heat stress during key periods of the breeding season.

Ultrasonography combined with hormonal assays is useful for evaluating heat stress in follicle development and hormone regulation. This method ensured accurate measurements of follicle size and reliable hormone profiles. However, this study also had several limitations. The sample size of 30 cattle and the duration of the experiment could be constraints on the findings. Wick et al. (2021) emphasized that the impact of environmental stressors may become evident in longitudinal studies with larger samples, which is also a limitation mentioned herein. Furthermore, although we did not consider reproductive markers, we did not control other factors, including nutrition, social stress, and air quality which could influence reproductive performance in real-life situations. The potential limitation of the present study may be due to the relatively small sample size and short period to monitor the long-term influences of HS on AI success in bovine species.

Further studies on the gene action governing heat stress tolerance in ruminants should be guided by the implications of Demir et al. (2022), who suggested that genetic variation in reproductive characteristics might determine the response to environmental stress. Additionally, there is a need for longitudinal investigations with larger sample sizes to evaluate the long-term effects of heat stress on reproduction over successive breeding seasons. Further research on the interaction of heat stress with other environmental factors, including feed, nutrition, and humidity, could help to better understand the putative factors that affect reproduction. In conclusion, exploring different heat stress amelioration methods (dietary additives, genetic improvement for some heat-resistant characteristics, new cooling methods) may be helpful in maximizing fertility in heat-challenged populations.

In summary, this study offers important information on the inhibitory effects of heat stress on the development of ovarian follicles and the levels of estradiol in large ruminants and contributes theoretically and practically to the area of animal reproductive physiology. Although long-term effects and strategies to alleviate them remain issues that need to be resolved in future studies, the current study paves the way for enhancing the reproductive performance of livestock under adverse environmental conditions.

5. CONCLUSION

The main findings of this study were that heat stress hampers ovarian follicle development and reduces estradiol concentrations in large ruminants with no effect on progesterone levels. These findings are valuable for understanding the physiological effects of heat stress on reproductive function and support the hypothesis that environmental stressors negatively affect ovarian function. These findings have important implications for animal reproductive physiology, especially for a better understanding of hormonal and follicular alterations because of heat stress. The findings of this study point to potential implications for minimizing heat stress in breeding animals, for example, through cooling systems or selective breeding for heat-tolerant breeds. However, several gaps still exist in literature, with long-term effects on fertility and the genetic basis of tolerance to heat stress among the most relevant. Further studies are needed to explore these findings, particularly longitudinal designs and genetic aspects of resilience to heat stress. This study is not without limitations, as the sample size was small and the study was of short duration which may limit the general ethical application of the findings. Subsequent research should overcome these limitations by using larger sample sizes and longer follow-ups to further expand the duration of exposure to occupational heat stress. In summary, this study offers important insights into the impact of heat stress on reproductive function in large ruminants and contributes to a further understanding of the physiological and endocrine alterations induced by environmental stress. Although further investigation is necessary, these findings offer essential insights to advance future developments in managing heat stress in livestock and improving reproductive efficiency in the context of climate change.

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