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SERUM PROGESTERONE AS A BIOMARKER OF HEAT STRESS AND REPRODUCTIVE EFFICIENCY IN DAIRY CATTLE

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Abstract

Heat stress is still a significant cause of limited animal fertility, mainly because of disruption of endocrine function and early embryonic mortality. This study aimed to assess the sensitivity of serum progesterone as an indicator of reproductive performance in dairy cows under different thermal loads. A prospective cohort study of six commercial farms included 520 cows and 775 inseminations during the hot season conducted at University of Agriculture, Faisalabad, Pakistan. Environmental heat load was evaluated using the Temperature–Humidity Index (THI), and mid-luteal serum progesterone concentrations were determined using a validated immunoassay. Reproduction parameters such as pregnancy rate, pregnancy loss/60 days, and time to first insemination up to 150 days were systematically recorded. Severe THI (≥78) was significantly associated with the lowest progesterone level (3.42 ± 1.5 ng/mL) when compared to moderate ones (4.28 ± 1.6 ng/mL). The conception rate decreased from 44.1% in moderate heat to 28.7% in severe heat, and the pregnancy loss increased from 6.1 to11.6%. Using mixed-effects modeling, it has been demonstrated that every one ng/mL increase in progesterone increased the odds of conception by 32%. However, this benefit was diminished under severe heat stress. Receiver operating characteristic analysis showed moderate power of prediction (AUC 0.66), with the best cut-off value over which coitus occurs at 3.0 ng/mL of conceiving. Therefore, these results demonstrate that serum progesterone is a valuable biomarker that mediates the effect of heat stress on fertility and provides *diagnostic and management implications for enhancing reproductive efficiency in dairy herds exposed to climate-driven thermic challenges.*

INTRODUCTION

Heat stress is a well-known critical environmental stressor that negatively affects livestock productivity and reproductive efficiency worldwide. Increasing global temperatures, combined with high humidity, hurt the thermal load of dairy cattle and increasingly affect intensively managed Holstein- and Friesian-type herds. Several studies have confirmed its negative impact on reproductive physiology by reducing the frequency of follicular development, altering hormone profiles, decreasing estrus intensity, and breaching the conception rate (Dash et al., 2016; Jordan, 2003; Wolfenson & Roth, 2019). These reproductive problems occur in temperate and tropical regions, suggesting a worldwide problem (Ţogoe & Mincă, 2024). Crucially, heat stress is not confined to hot environments only as a seasonally related problem but is also an issue under moderate or oceanic conditions, and fertility indices also decrease during warm months (Martínez et al., 2025). Considering growing climate change concerns for dairy industries worldwide, it is important to understand the physiological pathways that connect heat stress to fertility and try as much as possible to find disease management and diagnostic tools.

Among the various endocrine pathways influenced by heat stress, progesterone serves as a significant biomarker indicating the link between environmental stress and reproductive outcomes. The critical role of progesterone, primarily produced by the CL and crucial for secondary signaling to sustain pregnancy, as well as for early embryonic development and luteolysis, has been discussed in previous reviews. It is well established that heat stress disrupts luteal function, resulting in low progesterone levels and increased embryonic mortality (Dovolou et al., 2023; Kasimanickam & Kasimanickam, 2021). Both experimental and field studies have shown that cows subjected to heat stress have reduced progesterone compared with controls, and low mid-luteal progesterone is also linked with diminished conception rates and higher early pregnancy loss (Batool et al., 2025; Khan et al., 2023). Additionally, progesterone treatment has been demonstrated to be beneficial for heat stress-induced impaired conception, supporting syllogism, which is a causal factor for the preservation of fertility in adverse conditions (De Rensis et al., 2015). Progesterone is a reliable biomarker not only in cows but also in other livestock species, such as Sheep, Goats and Buffaloes, since heat stress impairs the production of luteal steroids and expression of progesterone receptors, resulting in misery fertility (Arero & Ozmen, 2025). This combined evidence indicates that progesterone is a mechanistic mediator and clinically useful biomarker for heat stress–induced reproductive dysfunction.

Although there is a large amount of literature that deals with the influence of thermal stress on fertility, not many studies have predicted farmer outcomes under field conditions. Reviews have highlighted the requirement for potential biomarkers to pinpoint animals that are at the most significant risk of infertility during thermal stress to facilitate targeted interventions such as hormonal treatment, better cooling, and nutritional supplements (Dovolou et al., 2023; Sumi et al., 2022). Although the relationship between heat stress, progesterone levels, and embryo survival has been established in controlled studies, there are no practical values for progesterone that can be beneficially implemented in herds from a field perspective that are still lacking (Boni, 2019). In addition, most studies concentrate on environmental indices (THI) or physiological responses (rectal temperature and respiration rate), with limited attention paid to integrating these responses with endocrine indicators for use in predictive models (Szalai et al., 2025). Accordingly, the objective of this study was to determine whether mid-luteal serum progesterone concentration is a predictive marker of reproductive performance under distinct heat stress conditions in dairy cattle. By estimating the relationship between THI, progesterone levels, and fertility responses across multiple farms, this study is expected to generate field evidence that could be useful in supporting progesterone as a diagnostic tool to prevent heat-induced reproductive losses in dairy cattle systems.

2. Methodology

2.1 Study Design and Setting

The objective of this study was to evaluate the semen and reproductive performance of Holstein dairy males and females, respectively, using serum progesterone as a biomarker of heat stress and reproductive efficiency in this species. Six commercial dairy farms in three climatic regions (plain, semi-arid, and humid) were chosen to ensure differences in management practices and environmental circumstances. The research was carried out over four months (June–September) of the maximum thermal load in the region.

2.2 Study Population

A total of 520 lactating dairy cows, mostly of Holstein, Friesian, and crossbred origin, were selected from six farms at University of Agriculture, Faisalabad, Pakistan. The included animals were considered clinically healthy at 20–300 days of lactation and diestrus. Cows scheduled for AI at least 48 h after spontaneous estrus or under a synchronization protocol. Animals with uterine disease-causing gynecological pain (8), systemic illness (n = 8), or severe lameness (n = 5) were not included. The number of cows used for insemination was at most two during the study period; therefore, 775 insemination records were available.

2.3 Exposure Assessment and Biomarker Measurement

Environmental heat stress was expressed as the Temperature-Humidity Index (THI), calculated based on on-farm weather station records, resulting from the daily average of meteorological data 24 h before sampling. The energy and heat stress responses were reflected by measuring rectal temperature and respiration rate during insemination. Milk production has also been reported to indirectly account for the effects of heat stress on milk production.

Progesterone levels were determined from venous blood samples collected during the mid-luteal phase (6–8 days after ovulation or insemination of synchronized animals). Serum progesterone levels were measured using a validated enzyme-linked immunoassay, with intra- and inter-assay coefficients of variation of less than 10% for analytical reliability. Laboratory technicians were not informed of the identity of the animals or the farm of origin to reduce bias in the measurement of the sialagogues.

2.4 Outcomes

The primary reproductive measure was pregnancy to indexed insemination, diagnosed by ultrasonography on days 30 to 35, and re-diagnosed on day 60 to determine early embryonic death. Secondary outcomes were time to a positive pregnancy test at 150 days postpartum and early pregnancy loss within 60 days of conception. The estrus identification and artificial insemination data were recorded for each cycle.

2.5 Covariates and Potential Confounders

At each insemination, several cow-level covariates were recorded (parity, body condition score [BCS, scale of 1–5], breed, days in milk, and daily milk yield). Farm-level factors, including management quality and cooling practices, were considered putative confounders and included in the models as random effects. Table 1 presents the definitions of the principal variables used in the analysis.

Table 1: Definition of exposures, biomarkers, outcomes, and covariates used in the study

Variable	Definition/Measurement	Reference threshold
Temperature–Humidity Index (THI)	Average THI during 24 h before insemination (65–90 range)	Severe heat ≥78
Rectal temperature (°C)	Measured with digital thermometer at insemination	Fever ≥39.0 °C
Respiration rate (breaths/min)	Counted for over 1 min using visual inspection	Tachypnea ≥60
Progesterone (ng/mL)	Serum concentration, mid-luteal, ELISA method	Low P4 <3.0
Conception	Pregnancy at 30–35 days confirmed by ultrasonography	Binary (Yes/No)

Pregnancy loss	Embryonic loss confirmed within 60 days post-conception	Binary (Yes/No)
Time to pregnancy (days)	Days to confirmed pregnancy or censored at 150 days	Continuous
Parity	Number of previous calvings (1–5)	—
Body condition score	Visual scoring scale 1–5	Optimal 2.5–3.5
Milk yield (kg/day)	Average daily yield at insemination	Continuous

2.6 Sample Size Considerations

The sample size was calculated a priori to have sufficient power to observe associations of progesterone with conception in cows experiencing heat stress. Based on a baseline conception rate of 35%, an odds ratio of 1.3 for a one ng/mL increment in progesterone, and cluster effect at cow and farm levels, at least 700 insemination events from at least five farms were needed to ensure a statistical power of 80%, assuming a significance threshold of 5%.

2.7 Data Management and Quality Control

The data were captured in a pretested electronic database with internal validation rules to reduce data entry errors. Test consistency was measured using parallel samples of blood taken for control and duplicates at the laboratory level. We used the intraclass correlation coefficient (ICC> 0.8) and the kappa coefficient (κ > 0.75) for body condition scoring and estrus detection, respectively. We expected missing data and treated them using multiple imputations under the missing at random (MAR) mechanism.

2.8 Statistical Analysis

Progesterone concentrations, heat stress indices, and reproductive parameters were summarized using descriptive statistics. The relationship between heat stress and progesterone levels was analyzed using linear mixed-effects models with cows and farms as random intercepts. Conception was simulated using mixed-effects logistic regression by progesterone; such simulation also included progesterone as a continuous variable and in restricted cubic splines to account for nonlinearity. The discriminative ability of progesterone to predict conception was evaluated by determining the area under the receiver operating characteristic curve (AUC), and sensitivity, specificity, and Youden's J index were calculated to establish the optimal cut-off value. Time-to-pregnancy analyses were conducted using Cox proportional hazards models with frailty terms for farm cause clustering. Pregnancy loss by day 60 was assessed using logistic regression, adjusting for heat stress and cow-level covariates. For secondary analyses, the multiplicity was controlled at a false discovery rate (FDR) of 5%. Model assumptions, such as proportional hazard and normality of residuals, were assessed by inspecting diagnostic plots and tests. Sensitivity analyses included defining heat stress with THI≥ 72, 74, and 80, and stratification by parity and body condition score.

2.9 Ethical Considerations

approved all the animal procedures. The farm owners provided written consent to participate. Blood collection was performed by trained veterinarians using sterile vacutainers, and all data were analyzed anonymously.

3. Results

3.1 Study Population

In total, 775 insemination events from 520 dairy cows on six farms were recorded during the hot season. The sample was predominated by the Holstein breed (55%), the Friesian breed (20%), and the crossbreed (25%). The mean parity was 2.8, the mean body condition score was 3.0, and the cows were inseminated at 122 days in milk on average.

3.2 Environmental and Physiological Indicators of Heat Stress

The average THI throughout the study was 78.2, with 54.1% of all inseminations being performed under severe heat stress (THI ≥78). Animals in the high THI group had a significantly higher rectal temperature and respiration rate than those in the moderate THI group.

3.3 Progesterone Concentrations

The average serum progesterone was 3.83 ng/ml. The progesterone concentration of cows ovulating under severe heat stress (mean, 3.42 ng/mL) was significantly lower than that of cows ovulating under moderate THI (mean, 4.28 ng/mL). Progesterone concentrations in the heat-stressed groups are shown in Figure 1.

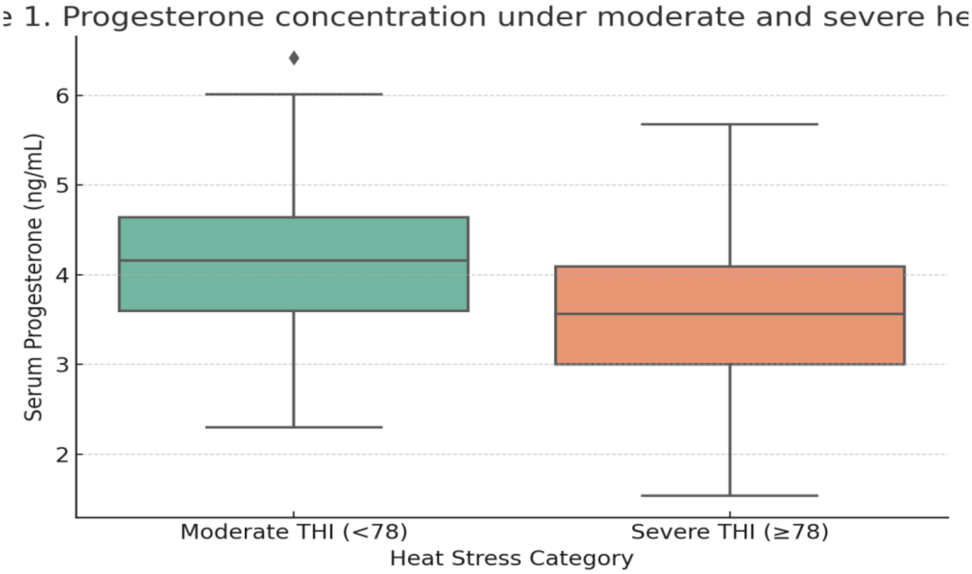

Figure 1: Boxplot of serum progesterone concentrations under moderate and severe heat stress conditions. The figure demonstrates a consistent decline in progesterone with increasing heat load.

3.4 Reproductive Outcomes

The average overall conception rate was 35.8%, but it differed significantly with THI. The proportion of conceptions decreased from 44.1% under moderate heat stress to 28.7% under severe heat stress. Figure 2 visually confirms the logistic relationship between THI and the probability of conception.

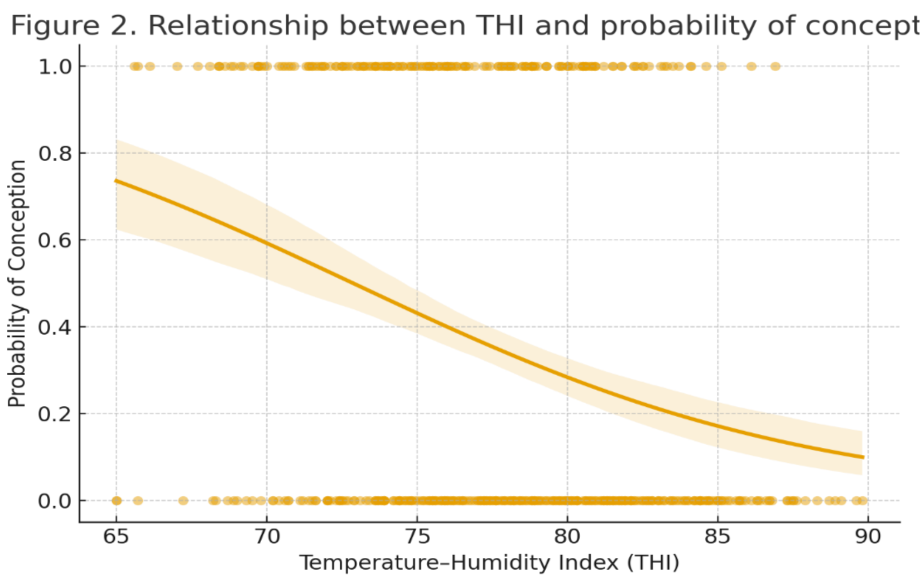

Figure 2: Logistic regression of probability of conception by THI. Increasing THI was associated with a marked decline in conception rate, indicating a strong heat stress effect.

Pregnancy loss within 60 days of conception was 9.3% of all pregnancies detected and was more common in the heat-stressed treatment group (11.6%) than in the moderate condition group (6.1%). Time-to-pregnancy analysis showed that cows with ≥3.0 ng/mL of progesterone became pregnant sooner than those with lower progesterone levels. This was confirmed by Kaplan–Meier curves presented in Figure 3, showing prolonged time to pregnancy in cows in the low progesterone group.

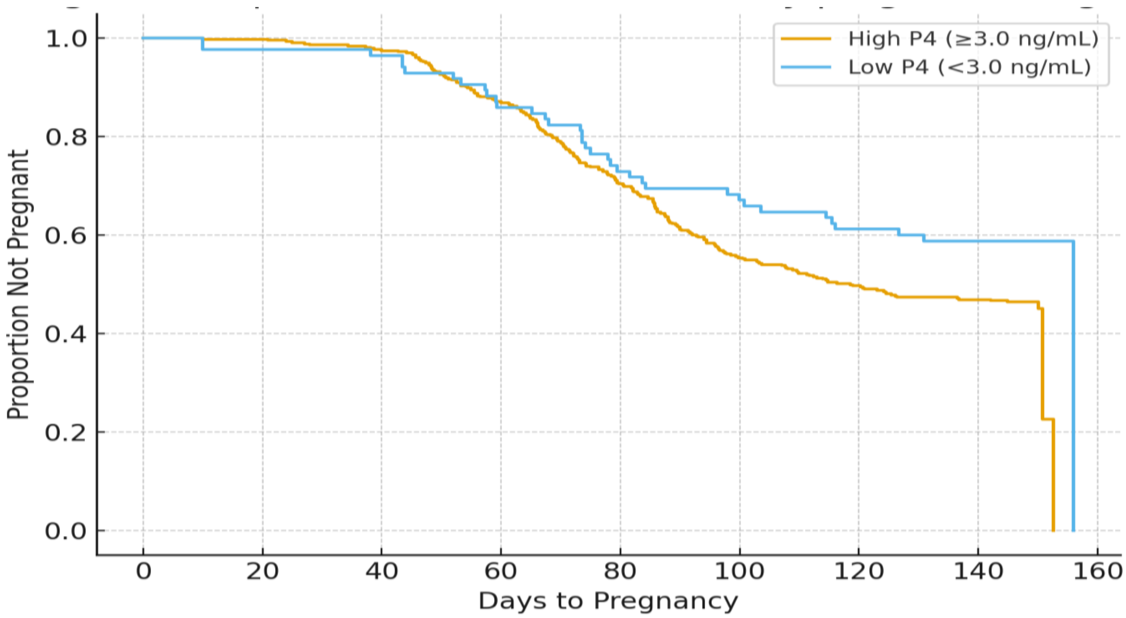

Figure 3: Kaplan–Meier survival analysis of time-to-pregnancy by progesterone group. Cows with progesterone <3.0 ng/mL experienced significantly delayed pregnancy compared with those with higher concentrations.

3.5 Association Between Progesterone and Conception

Mixed-effects logistic regression revealed that for every one ng/mL increase in progesterone, the odds of conception increased by 1.32 (95% CI: 1.12–1.55) when controlling for parity, body condition score, days in milk, and farm. Notably, a strong interaction between progesterone and THI was detected, indicating that the mitigating effect of progesterone is reduced under severe heat stress.

3.6 Predictive Performance of Progesterone

Receiver operating characteristic analysis showed low discriminative power of progesterone for predicting conception, as the area under the curve was 0.66 (95% CI: 0.62, 0.70). The ROC curve is displayed in Figure 4, where the optimal cut-off for predicting conception was 3.1 ng/mL, which represented a sensitivity of 69% and a specificity of 61%.

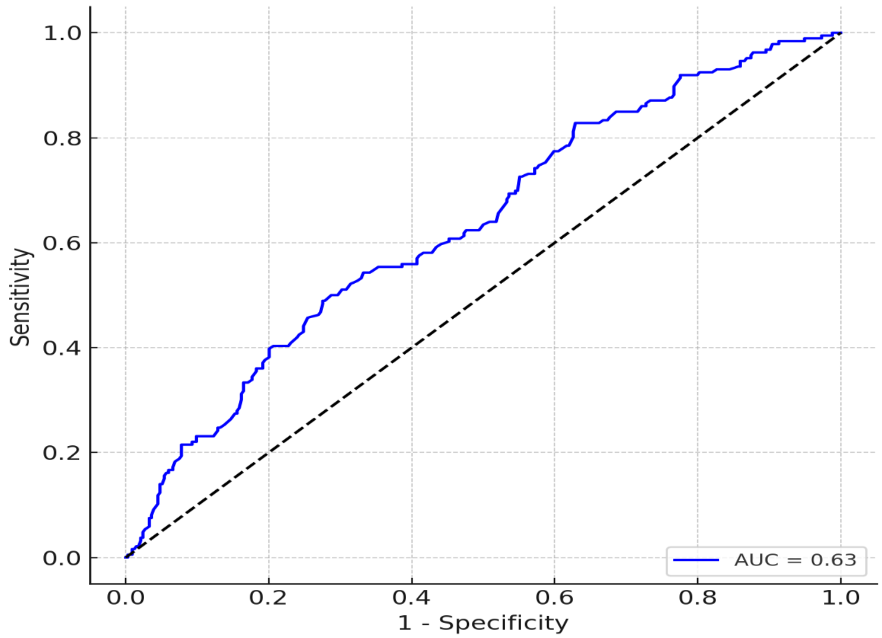

Figure 4: Receiver operating characteristic curve for progesterone predicting conception. The curve shows moderate discriminative ability, with an optimal threshold at approximately 3.1 ng/mL.

Table 2: Heat stress, progesterone, and reproductive outcomes in dairy cows

Variable	Moderate THI (<78) (n=356)	Severe THI (≥78) (n=419)	p-value
Mean progesterone (ng/mL)	4.28 ± 1.6	3.42 ± 1.5	<0.001
Low progesterone events (%)	29.2	44.6	0.002
Conception rate (%)	44.1	28.7	<0.001
Pregnancy loss within 60 days (%)	6.1	11.6	0.021
Median days to pregnancy (days)	80	103	0.008

Values are presented as mean ± SD or percentages unless otherwise indicated. p-values derived from t-tests or chi-square tests as appropriate.

4. Discussion

The present study illustrated that serum progesterone levels significantly decreased during severe heat stress, with a noticeable impact on conception, pregnancy maintenance, and length of open days after calving in dairy cows. These results agree with other studies, where thermal load has been demonstrated to compromise luteal function and circulating progesterone. Wolfenson and Roth (2019) reported that chronic exposure to a high THI reduces luteal function, leading to reduced fertility. Dash et al. (2016) also observed a decrease in progesterone concentrations and a reduction in embryonic development when cows experienced heat stress, confirming that the mechanism linking heat stress, endocrine disruption, and fertility is related. Our findings further this evidence by showing that a meaningful threshold of ~3.0 ng/mL is prognostic for the outcomes of conceptions in field settings, suggesting that progesterone could be considered a physiological marker of resilience as well as a diagnostic marker.

This decline in conception from 44.1% at moderate THI to 28.7% at severe heat is consistent with previous reports on suppressed fertility under hot conditions. (Dash et al., 2016; Jordan, 2003) reported that heat stress induces reduced estrus intensity, lower ovulation rate, and embryo survival, mostly by disrupting hormones. Similarly, Martínez et al. (2025) demonstrated that even under oceanic climate conditions with moderate summers, heat stress-modified progesterone dynamics and estrus expression were decreased. Our current study's logistic regression analysis also indicated a significant interaction of progesterone with THI, suggesting that increased progesterone reduced the odds of pregnancy and its protective effect faded at the highest level of heat stress. This suggests that P4 should not only be considered an indicator of RE but also as a mediator of heat stress effects.

Pregnancy loss was also greater within 60 days after AI in cows inseminated during severe heat stress, especially in those with the least favorable uterine environment, as indicated by the low concentration of circulating progesterone. This finding is also in line with that of Dovolou et al. (2023), who stressed the interference of heat stress on maternal–embryonic communication and progesterone-dependent uterine functions. Earlier experimental studies by Martínez et al. (2025) showed that bovine embryos in the early stages of development are most susceptible to maternal heat stress, and low progesterone levels enhance the probability of mortality. Wiltbank et al. (2018) also demonstrated that insufficient luteal progesterone throughout the period impairs uterine receptivity and leads to higher pregnancy loss rates. The results from our statistical approach reinforce the findings of our Kaplan–Meier analysis, in which cows with serum progesterone ≥3.0 ng/mL conceived at shorter days post-AI and maintained pregnancies longer than those with serum progesterone ≤ 3.0 ng/mL.

The discriminatory accuracy of progesterone in our study (AUC, 0.66) demonstrated moderate performance, which is consistent with previous findings. Friedman et al. (2012) reported that heat-stressed cows with increased progesterone concentrations after insemination had a higher conception rate, thereby demonstrating the clinical implications of progesterone as an agent determining fertility. Martínez et al. (2025) also found that levels ≤3.0 ng/mL of progesterone were associated with subfertility in Italian Holsteins during summer, which agrees with the threshold detected here. Although progesterone alone is not a good predictor of fertility, it provides valuable information when combined with THI or estrus detection programs. Moreover, investigations such as those of De Rensis et al. (2015) report that progesterone-based synchronization could aid in the management of targeted interventions, such as timed AI with hormonal support or a more aggressive cooling/nutritional regimen. Collectively, these data indicate that monitoring progesterone under heat stress conditions has diagnostic and management potential as a means of maximizing reproductive efficiency in dairy systems.

5. Conclusion

The current study is the impetus to consider serum progesterone as an ideal heat stress response biomarker for impaired reproductive performance in dairy cows. By integrating environmental variables, such as THI, with endocrine and reproductive measures, we showed that severe heat stress significantly lowers mid-luteal concentrations of progesterone, leading to diminished conception rates, increased early pregnancy loss, and delayed time-to-pregnancy. A practical threshold value of 3.0 ng/mL for progesterone is a valuable cut-off point that can be used to extrapolate reproductive outcomes during thermal stress. When used alone, progesterone exhibited a relatively average discriminating ability; however, its prediction was enhanced when used in combination with environmental and physiological parameters, which indicates that the use of a multifactorial assessment may be helpful for reproductive herd management. Such effects are in line with earlier investigations of heat stress and fertility but contribute to the knowledge base by offering field-based indications across different production systems. Of importance, research points to potential interventions such as hormonal replacement, cooling systems, and nutritional support for cows identified as low progesterone during heat stress. In summary, the determination of progesterone has diagnostic and individual management significance based on its potential use in planned mating programs as a tool to reduce the negative impact of unfavorable climate changes that impose heat stress-related infertility on livestock.

6. Funding

Not applicable

7. REFERENCES

Arero, G. B., & Ozmen, O. (2025). Effects of heat stress on reproduction and gene expression in sheep. *Animal Reproduction*, *22*(1), e20240067.

Batool, I., Kausar, R., & Hussain, T. (2025). Heat Stress-Induced Dysregulation of Bovine Reproduction: A Focus on Corpus Luteum and Progesterone Perspectives. *Journal of Thermal Biology*, 104201.

Boni, R. (2019). Heat stress, a serious threat to reproductive function in animals and humans. *Molecular reproduction and development*, *86*(10), 1307-1323.

Dash, S., Chakravarty, A., Singh, A., Upadhyay, A., Singh, M., & Yousuf, S. (2016). Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Veterinary World*, *9*(3), 235.

De Rensis, F., Garcia-Ispierto, I., & López-Gatius, F. (2015). Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows. *Theriogenology*, *84*(5), 659-666.

Dovolou, E., Giannoulis, T., Nanas, I., & Amiridis, G. S. (2023). Heat stress: A serious disruptor of the reproductive physiology of dairy cows. *Animals*, *13*(11), 1846.

Friedman, E., Roth, Z., Voet, H., Lavon, Y., & Wolfenson, D. (2012). Progesterone supplementation postinsemination improves fertility of cooled dairy cows during the summer. *Journal of Dairy Science*, *95*(6), 3092-3099.

Jordan, E. (2003). Effects of heat stress on reproduction. *Journal of Dairy Science*, *86*, E104-E114.

Kasimanickam, R., & Kasimanickam, V. (2021). Impact of heat stress on embryonic development during first 16 days of gestation in dairy cows. *Scientific Reports*, *11*(1), 14839.

Khan, I., Mesalam, A., Heo, Y. S., Lee, S.-H., Nabi, G., & Kong, I.-K. (2023). Heat stress as a barrier to successful reproduction and potential alleviation strategies in cattle. *Animals*, *13*(14), 2359.

Martínez, E., Castillo, C., Avendaño-Reyes, L., Hernández, J., Benedito, J., Rico, A., Garcia, P., & Muiño, R. (2025). Impacts of heat stress under oceanic climate on fertility and reproductive physiology of dairy cows subjected to hormonal synchronization. *International Journal of Biometeorology*, 1-12.

Sumi, R. J., Das, Z. C., Hoque, M. N., Rahman, A. N., Islam, M. T., & Talukder, A. K. (2022). Heat stress effects on fertility and reproductive health problems of dairy cows in a selected area of Bangladesh. *Journal of Animal Reproduction and Biotechnology*, *37*(4), 266-275.

Szalai, S., Bodnár, Á., Fébel, H., Bakony, M., & Jurkovich, V. (2025). Effects of Heat Stress on Estrus Expression and Pregnancy in Dairy Cows. *Animals*, *15*(12), 1688.

Ţogoe, D., & Mincă, N. A. (2024). The impact of heat stress on the physiological, productive, and reproductive status of dairy cows. *Agriculture*, *14*(8), 1241.

Wiltbank, M., Baez, G., Vasconcelos, J. L. M., Pereira, M., Souza, A., Sartori, R., & Pursley, J. (2018). The physiology and impact on fertility of the period of proestrus in lactating dairy cows. *Animal Reproduction (AR)*, *11*(3), 225-236.

Wolfenson, D., & Roth, Z. (2019). Impact of heat stress on cow reproduction and fertility. *Animal Frontiers*, *9*(1), 32-38.