

# Impact of seasonal variation on endocrine profile during an estrous cycle in Jersey crossbred dairy cows

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**Abstract:** The objective of the study was to investigate the influence of season *viz.*, summer, winter, and isothermic (spring and autumn season), on endocrine profile in dairy cows. The daily temperature-humidity index (THI) was recorded using a dry and wet-bulb thermometer to envisage its variation among different seasons. For analysis of endocrine profile, blood samples were collected from thirty dairy cows (n=10 in each season) at 5-day interval i.e. day 0 (day of estrus) to day 20, of the estrous cycle for ELISA based estimation of estradiol-17 $\beta$ , progesterone, and cortisol. In results, the THI varied significantly ( $p<0.01$ ) in different seasons with maximum THI in the summer (72.54), followed by the isothermic (64.7) and lowest in the winter season (54.12). Among the endocrine profile, the plasma cortisol levels had a significant variation ( $p<0.05$ ) in different seasons with increased levels in the winter followed by the summer season, however, differences were non-significant ( $p>0.05$ ) in plasma progesterone and estradiol-17 $\beta$ . In summary, seasonal variation barely led to significant changes in the reproductive hormones (estrogen and progesterone) but alleviated cortisol levels during summer and winter season had been suggestive of stress and could result in reduced fertility.

**Keywords:** Cortisol; Estradiol-17 $\beta$ ; Progesterone; Temperature-humidity index

## Introduction

Dairy cows living in tropical climates are subjected to severe weather conditions including extended periods of high ambient temperature and sun radiation. High ambient temperature also affects the reproductive hormone profiles of cattle. The endocrine system is involved in animal adaptation to stress and it has a significant impact on circulating hormones (Johnson and Vanjonack 1976). Because of the neuroendocrine response to climatic variables, climatic factors or seasonal fluctuations have a significant impact on animal behavior, impacting animal production and health (Sejian et al. 2013). The pituitary, thyroid, and adrenal glands play an important role in acclimatizing dairy cattle to environmental stress through various thermoregulatory and metabolic functions (Johnson et al. 1988). Cows under heat stress secrete higher levels of progesterone. The adrenal gland acts as a source of this progesterone. This level of progesterone inhibits the LH surge of estrus and prevents ovulation (Nazafi et al. 2003).

There is a reduction in the concentrations of estrogen and inhibin from the dominant follicle which results in the inhibition of pituitary FSH secretion. Summer had lower levels of estradiol in follicular fluid than winter while comparing the first-wave dominant follicles on day 7 of the cycle (Wolfenson et al. 1997). Cortisol, prolactin, and thyroxin are other hormones whose levels are altered during different seasonal conditions. Activation of the hypothalamo-pituitary-adrenal axis during stress reduces the pulsatility of GnRH/LH by actions at both the hypothalamus and pituitary gland, ultimately depriving the ovarian follicle of adequate LH support (Phogat et al. 1997), thus, the cow fails to maintain estrous cycles and consequently lead to anestrus. This result in the loss of integrity of the granulosa cells and subsequently the oocyte competence, although estrus and fertilization may occur, the conceptus fails to develop into a pregnancy (Dobson and Smith 2000). Keeping in view these aspects, the objective of study was to investigate the seasonal effects *viz.*, summer, winter, and isothermic (spring and autumn season) seasons, on endocrine profile in dairy cows.

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## Materials and Methods

This study was conducted at Livestock Farm Complex, CSKHPKV, Palampur (Zone-2 of agroclimatic zones of Himachal Pradesh; Mid-hills and sub-humid zone), from May 2021 to April 2022 for a period of twelve months comprising three seasons including summer (May to August), winter (November to February), and isothermic season i.e. spring (March and April) and autumn (September and October) season. The daily mean values of wet and dry bulb temperatures at 12:00 noon were recorded from a wet and dry bulb thermometer placed outside the animal shed. THI was calculated according to the equation reported by NRC

$$THI = 0.72 \times (Tdb + Wdb) \text{ } ^\circ\text{C} + 40.6$$

Tdb = Dry bulb temperature ( $^\circ\text{C}$ )

Wdb = Wet bulb temperature ( $^\circ\text{C}$ )

Thirty cows (Jersey crossbred; n=10 in each season) kept under standard feeding conditions, under natural light, housed in dry concrete sheds, and milked twice daily were selected for the study. Cows were clinically healthy and normal cyclic, with no history of reproductive abnormality. Blood samples were collected from the jugular vein, on day 0 (day of estrus), 5, 10, 15, and day 20 of the estrous cycle in each season from all the animals for estimation of hormonal (estradiol-17 $\beta$ , progesterone, and cortisol) attributes. From each cow, 10 ml of blood was collected in a heparinized vial and centrifuged at 3000 rpm for 10 minutes for the separation of plasma. The plasma samples were stored at  $-20^\circ\text{C}$  until further analysis.

All the hormones were estimated from stored plasma samples ( $-20^\circ\text{C}$ ) after thawing them at room temperature. ELISA kits were used to analyze different hormones. Progesterone was estimated using DRG instrument GmbH, Germany ELISA kit, estradiol-17 $\beta$  using BT LAB Bovine Estradiol ELISA kit, and cortisol using BT LAB Bovine Cortisol ELISA kit. The estimations were carried out in TECAN SUNRISE Microplate Absorbance Reader (TECAN Austria GmbH, Austria) present in the Department of Veterinary Gynaecology and Obstetrics.

The recorded data were statistically analyzed using one-way ANOVA and with NCSS 2020, USA (Version 22.0.4) software.

## Results and Discussion

In our study, the temperature-humidity index was significantly different ( $p < 0.01$ ) in different seasons with the summer season having a higher THI followed by the isothermic and winter seasons (Table 1). The temperature-humidity index varied significantly in the three seasons but not up to an extent that would result in heat stress in cattle (Morton et al. 2007; Schuller et al. 2014).

A significantly higher ( $p < 0.01-0.05$ ) value of plasma cortisol was recorded during winter as compared to the summer and isothermic seasons on days 10, 15, and 20 of the estrous cycle (Table 2). These findings were in agreement with Titto et al. (2012) who reported a significant increase in plasma cortisol levels during winter in dairy cows. On the contrary, Dobson and Smith (2000) and Ahmed and Abdalla (2012) reported a rise in plasma cortisol levels in the summer season in dairy cows as compared to the

**Table 1:** Average values of dry bulb temperature, wet bulb temperature, and the temperature-humidity index (THI) during summer, winter and isothermic season (Mean  $\pm$  S.E)

Season	Dry bulb temp. ( $^\circ\text{C}$ )	Wet bulb temp. ( $^\circ\text{C}$ )	THI
Summer	26.25 $\pm$ 1.0 <sup>x</sup> (24.06-27.96)	18.11 $\pm$ 1.1 <sup>a</sup> (14.96-19.87)	72.54 $\pm$ 0.11 <sup>x</sup>
Winter	14.82 $\pm$ 1.31 <sup>y</sup> (13.96-18.36)	3.95 $\pm$ 1.5 <sup>c</sup> (0.93-8.03)	54.12 $\pm$ 0.34 <sup>z</sup>
Isothermic	21.96 $\pm$ 1.22 <sup>x</sup> (18.58-23.83)	11.5 $\pm$ 2.01 <sup>b</sup> (6.8-16.6)	64.7 $\pm$ 0.38 <sup>y</sup>

<sup>x,y,z</sup> Values with superscripts in same column differ significantly ( $p \leq 0.01$ )

<sup>a,b,c</sup> Values with superscripts in same column differ significantly ( $p \leq 0.05$ )

**Table 2** Values of plasma cortisol (ng/ml) in dairy cows at different days of estrous cycle during summer, winter and isothermic season (N=30) (Mean  $\pm$  S.E)

Day of estrous cycle	Summer	Winter	Isothermic
Day 0	4.17 $\pm$ 0.61	4.52 $\pm$ 1.23	3.46 $\pm$ 0.89
Day 5	4.16 $\pm$ 0.35	6.02 $\pm$ 2.51	2.62 $\pm$ 0.22
Day 10	3.76 $\pm$ 0.41 <sup>y</sup>	5.60 $\pm$ 0.75 <sup>x</sup>	2.20 $\pm$ 0.35 <sup>y</sup>
Day 15	4.35 $\pm$ 0.40 <sup>b</sup>	5.77 $\pm$ 0.92 <sup>a</sup>	2.69 $\pm$ 0.47 <sup>b</sup>
Day 20	4.34 $\pm$ 0.42 <sup>b</sup>	5.09 $\pm$ 0.92 <sup>a</sup>	1.94 $\pm$ 0.1 <sup>b</sup>

<sup>a,b,c</sup> Values with superscripts in same row differ significantly ( $p \leq 0.05$ )

<sup>x,y,z</sup> Values with superscripts in same row differ significantly ( $p \leq 0.01$ )

**Table 3:** Plasma progesterone (ng/ml) concentrations in dairy cows at different days of estrous cycle during summer, winter and isothermic season (N=30) (Mean  $\pm$  S.E)

Day of estrous cycle	Summer	Winter	Isothermic
Day 0	0.48 $\pm$ 0.1	0.49 $\pm$ 0.13	0.46 $\pm$ 0.03
Day 5	1.96 $\pm$ 0.38	1.90 $\pm$ 0.18	2.56 $\pm$ 0.41
Day 10	4.02 $\pm$ 0.43	4.33 $\pm$ 0.83	4.78 $\pm$ 0.62
Day 15	3.58 $\pm$ 0.46	4.00 $\pm$ 0.34	4.97 $\pm$ 0.6
Day 20	0.95 $\pm$ 0.14	0.83 $\pm$ 0.16	0.70 $\pm$ 0.09

**Table 4:** Plasma estradiol-17 $\beta$  (pg/ml) concentrations in dairy cows at different days of estrous cycle during summer, winter and isothermic season (N=30) (Mean  $\pm$  S.E)

Day of estrous cycle	Summer	Winter	Isothermic
Day 0	4.3 $\pm$ 0.05	3.67 $\pm$ 0.88	5.59 $\pm$ 1.32
Day 5	2.46 $\pm$ 0.33	2.36 $\pm$ 0.46	2.44 $\pm$ 0.04
Day 10	2.65 $\pm$ 0.48	2.47 $\pm$ 0.67	2.85 $\pm$ 0.34
Day 15	2.52 $\pm$ 0.52	2.81 $\pm$ 0.45	2.91 $\pm$ 0.22

winter and rainy seasons. This variation noted by different workers may be due to the climatic conditions prevalent in their area of work.

The cyclic pattern of plasma progesterone concentration recorded in our study (Table 3) was in agreement with known changes in corpus luteum function in cows that occur during the estrous cycle (Schomberg et al. 1967; Hafez 2008).

The plasma progesterone concentration recorded in our study was lowest at day 0 (day of estrus), reaches a peak at the mid-luteal phase, and then starts to decrease with declining corpus luteum and was in agreement with Hafez (2008). However, no significant difference was recorded for plasma progesterone concentrations in different seasons (Table 3).

The peak levels of estrogen were achieved during the last part of the estrous cycle which is during proestrus which may be due to the presence of preovulatory follicle development (Alvarez et al. 2000; Hafez 2008; Naik et al. 2013).

During the start of the estrous cycle, in the phase of estrus, the estrogen concentration starts decreasing significantly from day 0 (day of estrus) to day 3 of the estrous cycle and causes ovulation in metestrus and initiates CL formation (Hafez 2008; Naik et al. 2013; Noakes et al. 2019). Later on, from the 3<sup>rd</sup> to 6<sup>th</sup> day, the concentration of estrogen increases significantly which is due to the first dominant follicle development (Alvarez et al. 2000; Naik et al. 2013). But after 6<sup>th</sup> day of the estrous cycle, the concentration decreases abruptly indicating dominant follicle atresia, developed during the first follicular wave (Hafez 2008; Noakes et al. 2019). However, in our study, no significant difference ( $p > 0.05$ ) was recorded between the plasma estradiol-17 $\beta$  concentration during summer, winter, and isothermic seasons (Table 4).

## Conclusions

Dairy cows were more prone to cold stress as compared to heat stress as increased values of plasma cortisol were recorded in the winter season followed by the summer season. In peroration, the seasonal variation barely led to significant changes in the ovarian steroid hormones (estrogen and progesterone), however, alleviated cortisol levels during summer and winter season had been suggestive of stress and could result in reduced fertility in the area under study.

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