Research article

DOI: 10.48165/ijar.2024.45.02.7

The Indian Journal of Animal Reproduction

The official journal of the Indian Society for Study of Animal Reproduction

ACS Publisher www.acspublisher.com

Year 2024, Volume-45, Issue-2 (December)

ISSN 0970-2997 *(Print)* ISSN 2583-7583 *(Online)*

Influence of Thermal Stress and Seasonal Variations on Basal and Post-Service Progesterone Profile in Postpartum Dairy Cows under the Humid Tropical Climate

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ABSTRACT

The present study estimates levels of progesterone (P4) and its seasonal variations intended to assess the influence of thermal stress on post-partum fertility of cows. Seasonal variations of basal and post-service P4 levels were compared with climatic variables and stress indicators in serum. Across seasons, basal and three post service interval P4 levels did not vary significantly, whereas the mean P4 level of post service intervals showed significant seasonal variation with the lowest during summer. Also, P4 levels showed significant seasonal variation in conceived animals with the highest value during the favourable season preceding summer. While basal P4 levels showed a significant negative correlation with ambient temperature, post service P4 levels did not show a significant correlation with climatic, physical, and biological parameters studied. It is concluded that even though ovaries are functional throughout the early post-partum period, seasons of thermal stress appear to suppress progesterone secretion and fertility performance of cross-bred dairy cows under the humid tropical climate.

Key words: Crossbred cow, Fertility, Progesterone, Season, Thermal stress.

How to cite: Kutty, C. I. (2024). Influence of thermal stress and seasonal variations on basal and post-service progesterone profile in postpartum dairy cows under the humid tropical climate. *The Indian Journal of Animal Reproduction, 45*(2), 39–43. 10.48165/ijar.2024.45.02.7

INTRODUCTION

Progesterone secreted by the corpus luteum (CL) during the oestrous cycle and gestation is essential for the establishment and maintenance of pregnancy (Gomez *et al*., 2018) until the completion of the predetermined term. Maternal recognition of pregnancy prevents luteolysis so that growth and secretory function of CL is maintained

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Received 17-03-2024; Accepted 24-07-2024

and the P4 level continues to increase among the cows maintaining pregnancy compared to those suffered embryonic loss (El-hamd *et al.* 2019). Thermal stress has been reported to reduce the length of oestrous cycle, together with a reduction of P4 secretion in cows and heifers (Dash *et al.,* 2016) and was found associated with the reduced secretory activity of theca and granulosa cells (Wolfenson *et al.,* 2000).

Macias-Cruz et al (2016) reported a significant reduction in the P4 concentration especially between days 8 and 14 of the oestrous cycle during summer compared to the autumn season, and was due to decreased functionality of CL, even though the oestrus and ovulatory activities were unaffected. Elevated ambient temperature adversely affected embryonic survival *in utero* and was mainly due to a reduction in the circulating P4 (Hansen, 2009). However, earlier studies on the effects of thermal stress on plasma P4 levels have generated contradictory data in dairy cows and ewes (Macias-Cruz *et al*., 2016). Hence the present study was carried out to document the influence of thermal stress on progesterone secretion, based on the seasonal pattern of basal and post service progesterone levels in early postpartum cross-bred cows under the hot humid condition.

MATERIALS AND METHODS

The study was conducted at Livestock Research Station, Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University in India. The dairy farm of the station is located at an altitude of 60-70 meters MSL, positioned at 11°21' N and 76°21' E latitude and longitude respectively. The herd consist of cross-bred cows were intensively managed with feeding and breeding protocols as per standard recommendations (ICAR-NIANP, 2013). Out of the cows calved each month, randomly selected four based on (a) uneventful completion of the initial fortnight postpartum, (b) belongs second to fifth parity, (c) optimum body condition score, and (d) history of regular milk yield in the previous lactation. The study was performed from day 28 to 91 post-partum replacing four cows every month so that only eight cows were under the study each month and continued year round from August to July.

The animals were subjected to B mode ultrasonography for detection of ovarian follicles and blood samples were collected by jugular venipuncture at regular weekly intervals. Cows inseminated during the study period were exempted from regular scanning and blood collection until their return to service or declared non pregnant through ultrasonographic detection from Day 25 post AI. Post service serum samples were collected from inseminated (33) cows on Day 7, 14, and 21 of AI. Serum samples were stored and tested for P4 and HSP 70 (heat shock protein 70) levels using ELISA kits; Pathozyme (Omega diagnostics® Ltd, U.K.) and Bovine HSP 70 (Chongqing Biospes Co Ltd, China) respectively. Climatic variables including ambient temperature and relative humidity were also recorded throughout the study period. Both basal and post service P4 levels of early post-partum period were compared between seasons such as SON (Sep-Oct-Nov being Northeast Monsoon), DJF (Dec-Jan-Feb being Post monsoon), MAM (Mar-Apr-May being Summer) and JJA (Jun-Jul-Aug being Southwest monsoon) prevailing in the region (Kutty, 2021) for the pattern of variation and correlation with biological stress indicators as well as climatic factors (Kutty *et al*., 2023) to assess the influence of thermal stress factors on serum progesterone levels.

RESULTS AND DISCUSSION

A total of 445 and 99 serum samples were stored being routine and post service samples respectively and analyzed to assess the basal and cyclical luteal phase levels of P4 during the early post-partum period across the four seasons and are illustrated in figure 1 and table 1 respectively. During the early post-partum period, the basal level of P4 in serum is contributed by the regressing corpus luteum of gestation and extra-gonadal sources. After first post-partum ovulation or luteinization of the follicles without ovulation, the P4 level elevates depending upon the secreting ability of such CL. Since basal and post service levels of P4 during the post-partum period vary widely depending upon many factors, both types of P4 were considered separately.

Among the 445 samples analyzed, basal P4 level ranged from 0.07 to 9.15 ng/mL with a man value of 1.63 ± 0.12 ng\ml. Across the seasons, the lowest level of basal P4 assessed was 0.36 ng/mL during MAM coinciding the period of maximum thermal stress and the highest 1.01 ng/mL was during JJA, even though the variation was non-significant between seasons. Between half years, the P4 level was significantly higher during rainy than non-rainy seasons. Basal P4 level was found negatively correlated ($P<0.05$) with maximum temperature and number of medium-sized follicles ($P<0.01$) in the ovaries. A decrease in P4 level with increase in AT indicates the adverse influence of thermal stress and it is in agreement with the earlier report of Abdalla *et al.* (2017). Similarly, the appearance of more medium follicles with decreased P4 level occurs due to withdrawal of progesterone block on follicle growth (Starbuck *et al.,* 2004).

Progesterone is highly essential for the manifestations of the oestrous cycle, conception, and maintenance of pregnancy, inverse relationship with ambient temperature can be attributed to cause infertility conditions associated with TS (De-Rensis *et al.,* 2017). A significantly high level of P4 was estimated during the rainy season and a positive correlation with relative humidity, indicates that besides ambient temperature other factors also regulate cyclical activity in these animals. A higher level of basal P4 ($P<0.05$) during the rainy season and negative correlation with maximum temperature indicates that thermal stress causes impairment of normal cyclical activity in animals, which is in agreement with the report by Dash *et al.* (2016). Between seasons, basal P4 had an inverse but non-significant relationship with HSP 70 levels as shown in Fig. 1.

The overall mean of post service P4 level showed slight increase between 7th day and 14th of the cycle irrespective of the seasons, even though the variation was statistically non-significant. However, between 14th and 21st day P4 level continued to increase only during two of the seasons. Mean P4 level of the three intervals showed significant variation ($P<0.05$) between seasons, the highest and lowest being during DJF and SON respectively. The mean values of P4 at each interval and the overall mean values were very low compared to the expected levels of (2.0 to 9.0 ng/mL) post service P4 in cattle (Gomez *et al.*, 2018) and are attributable to the persistence of higher ambient temperature and /or humidity causing thermal stress throughout the year (Kutty, 2021) as well as herd differences. Earlier studies also reported a decrease of post service P4 levels among animals under stress than non-stressed animals (Macias-Cruz *et al.,* 2016).

Between the three post-service intervals, there was a continuous increase from day 7 to day 14 attributable to the functional CL present at this stage irrespective of conception. Mean P4 level showed slight elevation at Day 14 and the increase continued even at Day 21 during SON and JJA. Corresponding to this observation, number of Regressing CL and size of the functional CL were lowest during SON and JJA (Table No 2), indicating minimal formation accessory CL, contributing to P4 secretion, during these two seasons. Whereas size of the functional

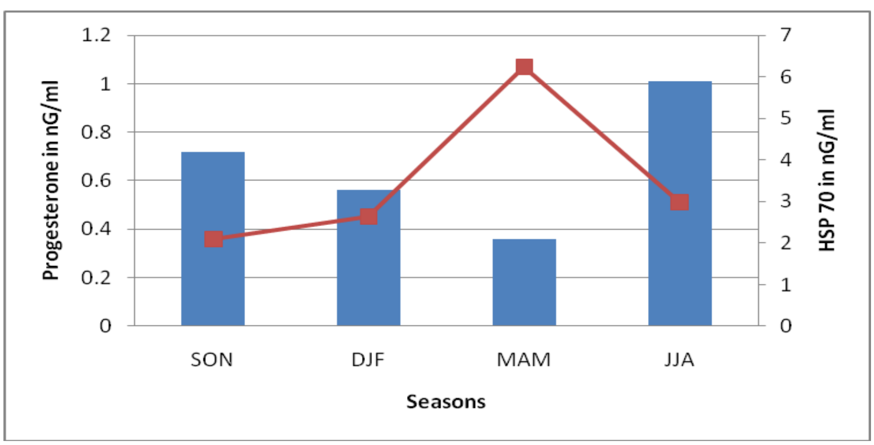

Fig. 1: Basal progesterone and HSP 70 levels in the serum during the early postpartum period compared across four different seasons

Table 1: Comparison of mean ± SE serum progesterone levels (ng/mL) during the three post-service intervals during different seasons

Season	Mean ± SE serum progesterone levels (ng/mL)							
	Day 7 (n=33)		Day 14 (n=33)		Day 21 (n=32)		Total (n=98)	
SON	10	0.59 ± 0.06	10	0.68 ± 0.05	9	0.73 ± 0.04	29	0.65 ± 0.03[a]
DJF	13	1.12 ±0.29	13	1.22 ± 0.34	12	1.19 ± 0.32	38	1.18 ± 0.18[b]
MAM	5	0.90 ± 0.31	4	1.47 ± 0.74	4	1.07 ± 0.28	13	1.13 ± 0.25[b]
JJA	5	0.52 ± 0.08	6	0.70 ± 0.06	7	0.92 ± 0.22	18	0.73 ± 0.09[a]
Total	33	0.84 ± 0.13[ns]	33	0.99 ± 0.16[ns]	32	0.97 ± 0.13[ns]	98	0.93 ± 0.08*
F-value (p-value)	1.376 (0.270)		1.186 (0.332)		0.590 (0.627)		3.124 (0.030)	

Ns-non-significant; * Significant (P<0.05); Means with different superscripts vary significantly; SON – Sept.-Oct.-Nov. – North east monsoon; DJF – Dec. - Jan. - Feb. - Post monsoon; MAM – Mar. Apr. - May – summer; JJA – June- July – Aug. – South west monsoon

Table 2: Mean ± SE of the numbers and size of functional and regressing corpus luteum across the seasons

Parameter	Details of CL during USG examination				
	SON	DJF	MAM	JJA	Overall
Number of FCL	12.33± 1.76	17.33±1.33	18.00±2.52	17.00± 3.79	16.17 ± 1.27
Size of FCL (mm)	17.43 ± 0.81	18.28 ± 0.69	17.98 ±0.41	17.57 ± 1.07	17.81± 0.35
Number of RCL	8.00 ±0.58	12.00± 1.00	10.33± 2.85	6.00± 1.73	9.18 ± 1.02
Size of RCL (mm)	10.16 ± 0.52	12.34 ± 0.59	11.40 ± 0.51	12.04 ± 1.23	11.48 ± 0.41

Variations non-significant between the seasons (P>0.05)

CL and number of regressing CL was more during DJF and MAM so that P4 level showed major elevation at Day 14, attributable to increased secretion by larger CL and more accessory CL during these seasons. However the P4 level dropped by Day 21, attributable to the luteolysis in non-conceived animals. The drop of P4 value at Day 21 was minimal during DJF attributable to more numbers of conceived animals (17), and the overall mean value to P4 across all seasons showed decrease mainly contributed by a major drop of P4 at Day 21 during MAM - the period of highest ambient temperature. Thermal stress causes follicular Co-dominance (El-Tarabany and El-Tarabany, 2015) leading to more accessory CL formation contributing more to P4 secretion and causes faster luteolysis and transient drop of P4 consequent to non-conception .

Across seasons, the P4 level of Day 14 was highest (1.47 ng/mL) during MAM as against higher levels of Day 7, Day 21, and the overall (1.12, 1.19, and 1.18 ng/mL, respectively) during DJF. During SON, P4 levels of Day 14, Day 21, and the overall had the lowest value than other seasons. This can be attributed to more non-conceived animals from MAM and JJA available in this season, so the mean P4 level remains low in agreement with the report by Gomez *et al.* (2018). However, basal and post service P4 levels did not show significant correlation with any of the weather parameters including THI, and HSP 70, except with the presence of a recent RCL in the ovary, which can be attributed to the adaptation out of continuous exposure to thermal stress over the years so that fertility is maintained across the seasons.

Out of the 33 inseminations done in the study animals, 17 resulted in conception. Comparison of P4 levels between conceived and non-conceived animals showed a significant (P<0.05) difference (1.10 ± 0.13 and 0.70 ± 0.03 respectively) and the season-wise values are shown in Table 2. Siregar et al. (2017) reported significantly higher levels of P4 in animals that maintain pregnancy (2.4 to 4.2 ng /mL) as against those who suffered embryonic death (1.93 ng/mL). Similarly, higher levels of serum P4 on days 6, 13, and 20 post-AI were reported in conceived animals by Gomez et al. (2018). Mean P4 levels among conceived animals showed highly significant (P<0.01) variation across seasons with higher levels during non-rainy (1.59 ng/mL) than rainy (0.72 ng/mL) seasons. Mean P4 levels of non-conceived animals also showed higher levels during non-rainy (0.71 ng/mL) than rainy (0.50 ng/mL) seasons, though the variation was non-significant.

Table 3: Post-service (Mean ± SE) progesterone levels (ng/mL) compared between conceived, not conceived, and total animals in four different seasons

Season	Mean ± SE serum progesterone levels (ng/mL)		
	Conceived (n=57)	Not Conceived (n=41)	Total (n=98)
SON	0.70 ± 0.04^a	0.61 ± 0.05	0.65^a
DJF	1.61 ± 0.34^b	0.79 ± 0.07	1.18^b
MAM	1.56 ± 0.41^b	0.62 ± 0.07	1.13^b
JJA	0.73 ± 0.09^a	0.49 ± 0.00	0.73^a
Total	$1.10 \pm 0.13^{**}$	0.70 ± 0.03^{ns}	$0.93 \pm 0.08^{*}$
F-value (p-value)	1.298 (0.009)	2.477 (0.980)	3.124 (0.030)

*** Significant (P<0.05) ** Significant (P<0.01) ns – non-significant**

Means with different superscripts varied significantly within the columns

Comparison of post-service P4 levels for conceived as well as total animals showed a similar seasonal pattern with significant variation having higher values during DJF and MAM than the other two seasons as shown in Table 2, whereas the variation was non-significant for non-conceived animals. While DJF formed the favourable season with respect to weather variables contributing to thermal stress, MAM was also found to have enhanced ovarian activity with more number of larger follicles contributed by follicular co dominance caused by thermal stress (El-Tarabany and El-Tarabany, 2015) and corresponding larger CL secreting more P4 as a supportive mechanism in conceived animals, concurring with the report of Macias-Cruz et al (2016) attributing variation of P4 levels to the secretory function of CL. Through impairment of P4 secretion by the luteal cells, thermal stress also caused reduced ovulation rate and affected CL formation causing reduced fertility during the warm climate (Lopez-Gatius *et al.*, 2005).

CONCLUSIONS

The basal P4 level of the post-partum period did not vary significantly between seasons. However, mean values of the post service P4 at three intervals varied significantly between seasons with the lowest during summer. Among conceived animals, P4 level was significantly higher during summer and was found to be associated with larger size of functional CL and more numbers of regressing CL attributable to the influence of thermal stress. Also, there was significant negative correlation of P4 level with maximum temperature indicating various adverse consequences of thermal stress on fertility.

ACKNOWLEDGEMENTS

The author is grateful to Dr. Thirupathy Venkatachalapathy, Head of Livestock Research Station, Thiruvazhamkunnu during the period of study, for the facilities provided. Technical guidance of Dr Bibin Becha B. and Dr. Abdul Azeez C. P. Assistant Professors, Department of ARGO during the study are thankfully acknowledged.

CONFLICT OF INTEREST

The author has no conflict of interest to declare.

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