Haryana Vet. (December, 2023) **62(2),** 6-9 ISSN 0033-4359 (P) **Research Article**

INFLUENCE OF CORTISOL LEVEL ON OVARIAN FOLLICULAR ACTIVITY IN POSTPARTUM SAHIWAL COWS DURING HOT-HUMID SEASON

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ABSTRACT

Present investigation was carried out to study the influence of circulating level of cortisol on ovarian follicular activity in postpartum Sahiwal cows during hot-humid months. Postpartum ovarian activity was monitored on alternate days beginning from any day between 60 and 90 postpartum in Sahiwal cows (n=16) up to next 21 days using ultrasound scanning to record the presence of dominant and/or ovulatory follicle (10 mm in diameter). Six animals (37.5 %) were classified as cyclic (follicle size 10 mm) between days 18 and 21 of the ultrasound scanning and out of these 6 cyclic animals, 3 animals expressed overt signs of estrus. Remaining 10 animals (62.5 %) were classified as acyclic. Serum cortisol concentration was estimated at weekly interval using radio-immuno assay kit and daily maximum temperature and relative humidity was recorded to calculate temperature-humidity index (THI). Non-significant differences were recorded in mean serum cortisol level between animals of cyclic and acyclic group on days 0, 7 and 14; however, significant ($P < 0.01$) higher level of serum cortisol was recorded on day 21 in animals of cyclic than acyclic group coinciding with the presence of dominant follicle in animals of cyclic group. Present study demonstrated a dramatic physiological increase in serum cortisol concentration around estrus phase. The circulating level of cortisol did not influence the growth and development of ovarian follicle in postpartum Sahiwal cows during hot-humid months and its level was not affected by THI value.

Keywords: Ovarian activity, Sahiwal cows, Seasonal stress, Serum cortisol, THI

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Seasonal stress is reported to activate the hypothalamo-pituitary-adrenal (HPA) axis in domestic farm animals resulting in increased synthesis and release of cortisol affecting the ovarian follicular development (Von Borell *et al.*, 2007). The increased cortisol levels could be due to the activation of hypothalamic-pituitary-adrenal axis and consequent increase in plasma glucocorticoids including cortisol (Marai *et al.*, 2007). Further, high level of gluco-corticoids during heat stress directly inhibits the meiotic maturation of oocytes (Gonzalez *et al.*, 2010) and corticotropic releasing hormone (CRH) inhibits the ovarian steroidogenesis, derived of the decrease in the secretion of luteinizing hormone (LH). The consequent decrease in estradiol results in reduced length and intensity of estrus expression (Masoumi, 2013).The direct effect of heat stress on the cow with altered endocrine regulation is due to impairment of hypothalamic-pituitary-ovarian axis (Ozawa *et al.*, 2005). Heat stress affects reproduction by inhibiting the synthesis of gonadotropin-releasing hormone and luteinizing hormone which are essential for estrus behaviour expression and ovulation. Heat stress influences the follicular development by reducing LH secretion (Gonzalez *et al.*, 2010), which disrupts the oocyte growth, reduces the growth of dominant follicles and increases the growth of subordinate follicles. In this perspective, present investigation was undertaken in post-partum acyclic Sahiwal cows with the hypothesis that ovarian follicular activity would be affected by presumed higher circulating level of cortisol amid seasonal stress during hot-humid months of June, July and August.

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MATERIALS AND METHODS

The present experiment was carried out on postpartum acyclic Sahiwal cows maintained at Bull Mother Experimental Farm of the institute during the month of June, July and August. Acyclic Sahiwal cows (n=16) between 60 and 90 days postpartum were randomly selected having history of normal parturition with no any palpable abnormality in their reproductive system. Ovarian activity was monitored through ultrasound scanning (Prosound ALOKA, Scanner 5-7.5 MHz trans-rectal probe) on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 21 to record the presence of dominant and/or ovulatory follicle (10 mm in diameter). Follicles were defined as non-echogenic (black) spherical structures with a clear demarcation between the follicular wall and antrum (Pierson and Ginther, 1987). The maximum diameters of ovarian follicles were measured using in-built electronic calliper. Based on findings of ultrasound scanning of ovaries, cows were divided in to two groups *viz*. cyclic and

acyclic. The cows of cyclic group showed presence of dominant and/or ovulatory follicle (10 mm diameter) whereas; the animals of acyclic group did not reveal presence of dominant and/or ovulatory follicle (< 10 mm diameter) during entire session of ultrasound scanning. Blood samples were collected from each cow at weekly interval on days 0, 7, 14 and 21 of ultrasound scanning to estimate serum cortisol concentration using commercial solid phase 125I radio immune assay (RIA) kit by employing standard technique (Meltzer *et al.*, 1983).

Meteorological data were obtained from Automatic Weather Station of Indian Meteorology Department installed at Krishi Vigyan Kendra, Anjora, Durg (CG). Information, consisting of daily maximum temperatures and relative humidity was used to calculate the temperature-humidity index (THI) for each day using the following equation as described by Kim *et al.* (2012).

$$THI = (0.8 \times Temperature) + [(\% RH/100) \times (Temperature - 14.4)] + 46.4$$

Where Temperature is the highest daily temperature in Celsius degrees and RH denotes the maximum relative humidity (%). Independent 't' test was used to compare mean serum cortisol level between cyclic and acyclic cow. The association between mean serum cortisol level and THI values on different days of observation was determined in a linear regression model using SPSS computer programme version 2018.

RESULTS AND DISCUSSION

The cyclic status of Sahiwal cows and mean serum cortisol concentration is presented in Table 1. Six cows (37.5 %) were classified as cyclic (follicle size 10 mm) between days 18 and 21 of the ultrasound scanning and remaining 10 cows (62.5 %) were classified as acyclic. Among cyclic cows, 3 cows expressed overt signs of estrus.

Non-significant differences were recorded in mean serum cortisol level between cyclic and acyclic group on days 0, 7 and 14; however, highly significant ($P < 0.01$) level of serum cortisol was recorded on day 21 in cyclic group coinciding with the presence of dominant follicle in cows of cyclic group. Mean serum cortisol concentrations were almost similar among the animals of acyclic group throughout the period of observation. Whereas, it was almost similar from days 0 to 14 but its level rose very sharply on day 21 in cyclic group. THI values ranged from 75.64 to 96.32 during experimental period. The regression analysis between mean serum cortisol level (dependent) and THI values (independent) on different days of observation during June, July and August is presented in Table 2, which revealed no any statistical relationship between these two variables suggesting no any influence of seasonal stress on circulating level of cortisol.

The density and volume of sweat glands in Sahiwal breed of cow has been reported to be greater than in that of Holstein Friesian cows in per unit of skin surface area (1058 glands/cm^2 vs. 920 glands/cm^2) and their crosses (709 and 691 glands/cm^2 in 75 and 87.5% HF crossbreds, respectively, Wang *et al.*, 2014. Cutaneous evaporation is the main avenue by which cattle dissipate heat via the involvement of sweat glands and other skin components. Therefore, with greater number of sweat glands/cm^2 of skin surface area, the heat tolerance in Sahiwal cows is greater than exotic breeds and crossbred cows. Furthermore, the total body surface area in relation to body size is greater in Sahiwal cows than exotic breeds of cow and their crosses owing to presence of hump and dewlap. These special anatomical features further help to dissipate the heat from body during summer season and thus helps in heat tolerance in Sahiwal cows. As mean serum cortisol concentrations were significantly lower and were almost similar in animals of both the groups than on day when dominant follicle was present in ovary of cyclic animals, it indicated that Sahiwal cows tolerated heat well and serum cortisol level did not increase despite higher THI values in animals of both groups.

On the contrary, among the cyclic cows (n=6), three exhibited overt signs of estrus and they recorded mean serum cortisol level as 13.63±0.48 ng/ml, whereas remaining three animals recorded its level as 10.61±0.24 ng/ml. The level of serum cortisol concentrations increased significantly with the presence of dominant follicle in cyclic animals in the preset study, which is consistent with observation of Lyimo *et al.* (2000) and Torres-Júnior *et al.* (2007) who reported that its cortisol level increased significantly during estrus.

The biological activity of cortisol correlates with the free protein-unbound concentration rather than with the total concentration, which includes both free as well as protein-bound fraction (Andersen, 2002). Free hormone theory applies well to significant increased level of cortisol with the presence of dominant follicle in present study. The ovaries express glucocorticoid receptors and are affected by cortisol, but lack the necessary enzymes for cortisol synthesis. Ovarian follicles modulate the biological activity of cortisol when follicular production of progesterone and 17 alpha-hydroxy-progesterone reach to levels that displace cortisol from its binding proteins, thus making it available for biological action. It may be suggested that with the availability of free cortisol in the

pre-ovulatory follicle just prior to ovulation, cortisol may function to reduce the inflammatory-like reactions occurring in connection with ovulation. This proposed theory attempts to justify why the serum level of cortisol is increased at the time of estrus with the presence of a dominant follicle (Anderson, 2000), as recorded in the present study.

Another explanation for rise in circulating level of cortisol during estrus phase is that the animals remain under physiological stress during the time of estrus that probably modulates the circulating concentration of cortisol (Lyimo *et al.*, 2000). These authors reported physiological rise in cortisol levels at the time of estrus to reach at its maximum level however, it did not exceed the physiological levels in rest situations.

Table 1. Mean serum cortisol concentration (ng/ml) in postpartum Sahiwal cows

Days of blood collection	Cyclicity Status		P value
	Cyclic (n=6)	Acyclic (n=10)	
0	7.2 ± 0.55^{aA}	7.38 ± 0.42^{aA}	0.40
7	7.21 ± 0.6^{aaA}	7.32 ± 0.51^{aA}	0.45
14	8.82 ± 1.17^{aA}	8.05 ± 0.74^{aA}	0.28
21	12.18 ± 0.67^{bB}	7.97 ± 0.52^{aA}	0.00011^{**}

Means within the column with different small letter (a, b) superscript differ significantly.
Means between the columns with different capital letter (A, B) superscripts differ significantly

Table 2. Mean serum cortisol concentration (ng/ml) in relation to THI during hot-humid season in postpartum Sahiwal cows

Days of observation	June			July			August		
	THI Value	Serum cortisol level	Regression P Value	THI Value	Serum cortisol level	Regression P Value	THI Value	Serum cortisol level	Regression P Value
0	95.58	7.102	0.1907	90.26	7.3003	0.9339	83.61	7.588	0.2390
7	91.00	7.611		82.63	7.3018		87.13	7.064	
14	87.72	8.7404		90.44	7.859		76.47	8.647	
21	93.67	8.116		86.04	9.114		77.25	11.50	

Non-Significant (P>0.05)

Present observation suggested that serum cortisol concentration is not influenced by THI values. Serum cortisol concentrations (ng/ml) were estimated in animals during hot-humid season with the hypothesis that level of serum cortisol might be influenced under heat stress conditions (Minton, 1994). However, finding of present study did not support this hypothesis as non-significant difference in serum cortisol concentrations between cyclic and acyclic animals indicated that its level is not influenced by heat stress. Similar to finding of present study, DuPreez *et al.* (2000) documented that plasma cortisol concentrations were lower in heat-stressed cows than in cooled cows. Singh *et al.* (2016) reported that mean plasma cortisol level significantly increased during winter season both in high and low yielding Sahiwal cows than during summer season. The possible explanation of finding of similar serum cortisol concentration both in acyclic and cyclic animals in present study might be that Sahiwal cows are well acclimatized with the climate and under heat stress their hypothalamo-pituitary-adrenocortical (HPA) axis was not activated in response to higher THI (Mete *et al.*, 2012 and Das *et al.*, 2014). However, contrary to present findings, Chandrabhan *et al.* (2012) reported significantly higher values of serum/plasma cortisol concentrations under heat stress in growing and adult Sahiwal cattle.

Therefore, from the findings of present study, it may be concluded that the circulating level of cortisol did not influence the growth and development of ovarian follicle in postpartum Sahiwal cows during hot-humid months. Serum cortisol level was not influenced by higher THI values in both cyclic and acyclic animals suggesting that Sahiwal cows had good heat tolerance property and circulatory concentration of cortisol may not be used as a marker for assessment of heat stress in Sahiwal cows.

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