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Mechanisms Linking Heat Stress to Poor Reproductive Performance of Nigerian Indigenous Zebu Cows

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ABSTRACT

Study was conducted to evaluate how heat stress affects follicular size, reproductive hormones, oestrus expression in different seasons. Twelve (*n*=12) zebu cows were utilized over a year. Ambient temperature, relative humidity was collected and THI value determined, rectal temperatures were also collected. Cows were synchronized for oestrus. Ultrasonic follicular studies were carried out until ovulation. Blood was collected to assayserum concentration of progesterone, oestradiol and Luteinizing hormones using Enzyme -Linked Immunosorbent Assay technique. Oestrus activities were monitored; follicular diameters at 42 hr were shorter *P< 0.05* in cold dry season than hot dry and raining season. At 72 hr follicular diameters were longer *P< 0.05* in hot dry season than cold dry and raining season. Time to peak of oestradiol was shorter *P< 0.05* in cold dry season than hot dry and raining seasons. Amplitude of oestradiol was higher *P< 0.05* in cold dry season than hot dry and raining seasons. Time to peak of LH surge was shorter *P< 0.05* in cold dry season, than hot dry and raining season. Amplitude of LH surge was higher *P< 0.05* in raining season than cold dry and hot dry season. Duration of LH surge was longer *P< 0.05* in cold dry season, than hot dry and raining season. Mounting was higher *P< 0.05* in cold dry season than raining and hot dry season. Conclusion: Heat stress has increased follicular size without increasing oestradiol concentration, reduced mounting, increased LH surge and progesterone concentration.

Keywords: Hormones, seasons, heat stress, zebu cows

The ambient temperature associated with infertility in cattle has drawn the attention of researchers all over the world (Armstrong, 1994). Several environmental factors play a crucial role in maintaining the reproductive function of the dairy cow (Nabenishi *et al.* 2011). Among these factors, heat stress has been identified to be the critical cause of infertility in dairy cattle (Nabenishi *et al.* 2011). Highly productive cattle are more susceptible to the change in the environment which is manifested as altered sexual cyclicity as well as the ovarian activity (Alves *et al.* 2014).

Cattle that are directly exposed to heat stress during summer season experience low fertility which is one of the constraints faced by farmers throughout the world (Lopez, 2003). This persisting effect of summer heat on fertility of cows results in altering the development of pre-ovulatory follicles during severe hot months (Wakayo *et al.* 2015). Even minor alterations in the core body temperature were established to be sensitive enough to induce changes in the oestrous cyclicity in dairy cows (Roth *et al.* 2001a). The altered oestrous cycle behaviour

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of cows exposed to heat stress results in late manifestation of oestrus and a lengthened oestrus interval which leads to a high incidence of silent oestrus/anovulation and anoestrus in dairy cows (Fisher *et al.* 2008).

Sexual behavior peculiarities of Zebu breed of animals, such as short length of oestrus and reduced mounting activity can lead to errors in oestrus detection (Piccione *et al.* 2003). Zakari *et al.* (1981) observed that intensity of oestrus behavior increased during the hottest months of the year and Galina *et al.* (1995) suggested that winter could limit the expression of oestrus in Zebu cows. Plasse *et al.* (1981) observed a high occurrence of long oestrous cycles and silent oestrus/anovulations during the winter, and Zakari *et al*. (1981) verified the occurrence of longer oestrous cycles in the pre-raining season than in the raining season in Zebu cows. Studies on oestrus behavior in Zebu cows can be made easier by oestrus synchronization. However, evidence suggests that the pharmacological agents utilized in synchronization can alter some animal oestrus behavioral characteristics, such as oestrus length (Plasse *et al.* 1981) and intensity (Orihaela *et al.* 1983).

Reproductive studies in Nigerian indigenous Zebu cowsby workers (Orituela *et al.* 1983; Dawuda *et al.* 1988; Pathiraja *et al.* 1988 and VohJr *et al.* 1989) have indicated poor reproductive performance of these Zebu cows. Knowledge of the mechanism(s) that are involved in causing this poor reproductive performance in our indigenous cattle will afford us with the opportunity to proffer solutions in solving the problem of infertility in these breeds of cattle.

It was therefore, hypothesized that there would be effect of environmental heat stress on the follicle size, profiles of reproductive hormones and oestrus expression in the indigenous cattle breeds. To test this hypothesis, the objectives of the current study were to evaluate the effects of heat stress on (i) follicle size (ii)reproductive hormones and(iii) oestrus expression.

MATERIALS AND METHODS

Study location

The study was conducted at the Veterinary Teaching Hospital Research Station, South Core, Federal University of Agriculture Makurdi Benue State, Nigeria. Makurdi is situated in the Southern Guinea Savannah, 600 metres above sea level on latitude 7° 14′North and longitude 8° 21′ East. The area has a warm temperatures ranging from 24 to 40 °C, with relatively greater temperatures occurring between March and May, the average rainfall is between 508 and 1016mm annually (Timi and Tor, 2016).

Determination of temperature humidity index (THI)

A year period of weather data (Ambient temperature and Relative humidity) were collected from the Air Force Base Weather Station located 2 kilometers away from the research station. The temperature humidity index (THI) was calculated for the three seasons using the formula developed by (Kible, 1964). It is as follows:

$$THI = 1.8 * Ta - (1 - HR) * (Ta - 14.3) + 32$$

Where: *Ta* = Mean Ambient Temperature in °C; *HR* = Mean Relative Humidity. The determined *THI* values were used to identify heat stress seasons in Makurdi and to examine the seasonal variation of THI.

Determination of Heat Stress base on THI values

Heat stress determined in zebu cows by Du Preez (2000) was adopted, THI value ≤ 74 is a thermal zone for zebu cattle, temperature humidity index (THI) values; (75-86) heat stress.

Experimental animals and management

The research was carried out with the approval of the Ethical Committee of the College of Veterinary Medicine, Federal University of Agriculture Makurdi, Nigeria. Twelve (n=12) matured, cycling, non-pregnant zebu cows with history of 2-3 successful calving, average weight of 338.92 ± 16.39 kg and body condition scores of 3.68 ± 0.13 on a scale of 5, were utilized over 3 replicate months (November-February, March-May and June-October). These breeds of cows were selected because they are widely distributed across the country in dense population. The cows were identified with the use of large plastic ear tags and kept for a 6 months period of stabilization, during which blood and fecal samples were

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collected to screen for parasites and treatment was instituted accordingly. Pregnancy examination using transrectal ultrasonography was carried out to ensure the cows were not pregnant. The cows were managed under semi-intensive management system by grazing them in the morning and supplemented with concentrate diet at 3% of their body weight in the evening. The concentrate consisted of cotton seed cake; maize and wheat bran in the ratio 1:2:4. Mineral salt licks and clean drinking water were provided *ad-libitum*.

Experimental Design

Repeated measure and randomized design model were used;this study was divided into three phases:

Phase I: November-February (cold dry season)

Phase II: March – May (hot dry season)

Phase III: June - October (raining season)

Monitoring of rectal temperature

Rectal temperature was monitored twice a week with the use of clinical thermometer between 12:00 PM -1:00 PM throughout the experiment period.

Oestrus synchronization

At the end of each phase,cows were synchronized foroestrus using $PGF_2\alpha$ (Synchromate that contained Cloprostenol manufactured by Bremer Pharma Gmbh 34414 Warburg Germany Batch No. 26176) at (500 μg) /cow intramuscularly. Two injections were administered 12 days apart.

Monitoring of oestrus activity

Oestrus was monitored 48 hr after administration of second dose of $PGF_2\alpha$ by visual observation of animals for signs of oestrus such as mounting,vaginal mucous discharge and hyperemia of the vulva for 2hr at each occasion twice daily between 06:00-08:00 AM and 04:00-06:00 PM, to determine oestrus intensity and duration. Oestrus intensity was the number of times the cows mounted other cows or stood to be mounted by other cows and oestrus duration was the length of time the cow was in oestrus.

Scoring of oestrus activities

Oestrus activities were scored using the methodology described by (Dupreez *et al*. 1990).

Follicular study

Ultrasonic follicular studies measuring the size of the largest follicles in first follicular wave using a real time B-mode Ultrasound scanner (manufactured by Edan Instrument Inc. 1019// Skeko Nashan Shenzhen 518067PR China with transrectal probe of 7.5 MHz linear array) was carried out ransrectally daily 24 hr after administration of second dose of $PGF_2\alpha$ until ovulation occurs. Occurrence of ovulation was considered as the presence of ovulation depression fossa. All cows ovulated with 18 hr.

Blood Sample collection

Blood sample for E2 assay commenced 24 hr after administration of second dose of $PGF_2\alpha$. Two (2) ml of blood samples through the indwelling catheter in the jugular vein were taken into a sample bottle without Ethylenediaaminetetraacitic acid (EDTA) to harvest sera at every 6 hr for 72 hr for oestradiol determination. Blood sample collection for LH also commenced 48 hr after administration of second dose of $PGF_2\alpha$ at the interval of every 2 hr for 72 hr for LH determination. Blood sample was collected twice a week for a period of 12 months for progesterone determination. Blood samples collected were kept at room temperature for 30 min and spun using centrifuge model 80-2 Lemfield Medical England at 3000 rpm for 15 sec and serum samples were harvested and stored at -20 °C until analysis.

Serum Hormonal assay

Progesterone, oestradiol and luteinizing hormones assay were carried out using Enzyme-Linked Immunosorbent Assay (ELISA) Kits (AccuBind, USA) according to the manufacturer instructions and ELISA Reader (Thermo Scientific Multi task an EX (Vantaa Find land) was used in this study.

Assays were validated using the absorbance (OD) of the calibrators for each hormone according to the manufacturer instruction. Inter- assay % CV was 14.5 and intra-assay % CVs 8.1. For progesterone, Inter- assay % CV was 12.5 and intra-assay % CVs 5.6 for oestradiol, and Inter- assay % CV was 13.6 and intra-assay % CVs 7.2 for LH.

Determination of Serum Proestrus Oestradiol (E_2) Surge

The proestrusoestradiol (E_2) profile were used to determine the serum proestrus E_2 amplitude (concentration), area under the E_2 secretion curve (duration), and time to E_2. Peak (area before curve). Proestrus E_2 surge was considered to have occurred if E_2 concentration in one of the thirty six consecutive six hourly serum samples was equal to or above 10 pg/ml following synchronized oestrus. This value was chosen because in all the animals that showed overt oestrus this value was the lowest elevated serum E_2 value 24 hr after synchronized oestrus. An E_2 surge was present irrespective of the peak values once it had exceeded 10 pg/mL following oestrus synchronization.

Determination of Serum Pre-ovulatory Luteinizing Hormone (LH) Surge

Serum pre-ovulatory luteinizing hormone (LH) profile were used to determine the LH surge characteristics, LH amplitude (concentration), duration of LH (area under the curve), and time to LH peak (area before curve). Preovulatory LH surge was considered to have occurred if LH concentration in one of the thirty six consecutive two hourly serum samples was equal to or above 2 ng/ml following synchronized oestrus. This value was chosen because in all the animals that had elevated LH concentration; this value was the lowest elevated serum LH value 24 hr after synchronized oestrus. An LH surge was present irrespective of the peak values once it had exceeded 2ng/L following oestrus synchronization.

Data for rectal temperature, progesterone, follicular diameters, surge characteristics for proestrusoestradiol surges and preovulatory-luteinizig hormone surges (time to peak, amplitude and duration)were analyzed by repeated measure ANOVA using R Studio (R Core Team 2019). Tukey's Honesty Significant Difference Test was applied to determine significant difference among the groups at $P < 0.05$.

RESULTS AND DISCUSSION

Temperature humidity index (THI)

Temperature humidity index (THI) values for the weather station were 45 in cold dry season, 93.4 in hot dry season and 93 in raining season respectively, Table 1. This result showed that zebu cows were not under heat stress in cold dry season. However, the THI values in hot dry and raining season showed that the cows were underheat stress.

Table 1: Ambient Temperature, Relative Humidity and Temperature Humidity Index values during cold dry, hot dry and rainy season

Season	Average Ambient Temperature (^{0}C)	Average Relative Humidity (%)	THI
Cold dry	35.8	45.8	45.0
Hot dry	37.5	65.0	93.4
Rainy	31.6	76.6	93.0

THI = Temperature Humidity index.

Cows rectal temperature during the cold dry, hot dry and raining season

The rectal temperatures were significantly higher $P<0.05$; in hot dry season (38.07 ± 0.03 °C and raining season 38.06 ± 0.03 °C respectively) than cold dry season (37.71 ± 0.04 °C; Table 2). There were no significant difference $P > 0.05$ between hot dry and raining seasons.

Table 2: Rectal temperatures of zebu Cows in cold dry, hot dry and rainy season

Season	Months	T °C
Cold dry	4	37.71 ± 0.04^b
Hot dry	3	38.07 ± 0.03^a
Rainy	5	38.06 ± 0.03^a

Keys: *n= No of cows*

a = significantly higher (P <0 .05) along the column.

b = significantly lower (P <0 .05)along the column.

Effect of heat stress on serum progesterone concentration

Serum progesterone concentration was significantly lower *P<0.05* in cold dry season (1.096 ± 0.075 ng/ml)than hot dry (1.408 ± 0.90 ng/ml) and raining seasons (1.361 ± 0.86 ng/ml, respectively; Table 3). There were no significant difference *P>0.05* between hot dry and raining seasons. This observation was consistent with the findings of (Rosenberg *et al.* 1977; Younas *et al.* 1993 and Ronchi *et al.* 2001) who reported that heat stress

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increases plasma progesterone concentrations. However was inconsistent with the findings of (Wilson *et al.* 1998; Roth *et al.* 2000; Guzeloglu *et al.* 2001) who reported that heat stress has no effect on the plasma progesterone concentrations during summer heat stress in dairy cows. These differences in progesterone concentrations could be due to breed difference and climatic condition in which the research was carried out.

Table 3: Serum Progesterone Concentrations of Bunaji and Bokoloji Cows Durig Cold Dry, Hot Dry and Raining Seasons

Season	Months	Plasma P_4 concentration (ng/ml)
Cold dry	4	1.096±0.08[b]
Hot dry	3	1.408±0.90[a]
Raining	5	1.361±0.86[a]

Keys: *n = number of cows*

a = significantly higher (P <0 .05) along the column

b = significantly lower (P <0 .05) along the column

Follicular dimensions

Follicular diameters at 24 hr after administration of second dose of $PGF_{2\alpha}$, were significantly longer *P<0.05* in raining season (8.62 ± 0.9 mm) followed by hot dry season (8.09 ± 0.52 mm) and then cold dry season (6.34 ± 0.68 mm). There were no significant differences *P>0.05* between hot dry season (8.09 ± 0.52 mm) and raining season (8.62 ± 0.92 mm), Table 4. At 48 hr, after administration of second dose of $PGF_{2\alpha}$, follicular diameters were significantly longer *p<0.05* in hot dry (10.75 ± 0.73 mm) followed by raining season (9.66 ± 0.92 mm) and then cold dry season (9.01 ± 0.45 mm); but did not differed significantly *P>0.05* between hot dry (10.75 ± 0.73 mm) and raining season (9.66 ± 0.92 mm). At 72hr after administration of second dose of $PGF_{2\alpha}$ follicular diameters were significantly longer *p<0.05* in hot dry season (17.01 ± 1.41 mm) followed by cold dry season (12.90 ± 1.22 mm) and then raining season (12.08 ± 0.82 mm). There were no significant differences *p>0.05* between cold dry (12.90 ± 1.22 mm)and raining season (12.08 ± 0.82 mm). Also, the follicular diameters were significantly longer *p<0.05* at 72 hr (12.08 ± 0.82 mm) followed by at 48 hr (9.01 ± 0.45 mm) and then at 24 hr (6.34 ± 0.68 mm) respectively. This observation corroborates the findings of (Bajagai, 2011; De Rensis *et al.* 2002) who reported that heat stress increases follicular size. However, it was inconsistent with the observation of (Shehab-El-Dean *et al.* 2010), who reported that heat stress decreases the diameter of follicles and induces biochemical changes in the follicular fluid. It is worth noting, however, that follicular size is not a good indicator of functional follicular dominance (Forttune *et al.* 1991). The follicles can be big for nothing if it lacks LH receptors that can trigger ovulation.

Table 4: Follicular diameter of zebu cows at 24, 48 and 72 hr after administration of second dose of $PGF_{2\alpha}$

	Follicular Diameters (mm)		
Time (hr)	Cold dry season n=12	Hot dry season n=12	Raining Season n=12
24	6.34 ± 0.68[bd]	8.09±0.52[bc]	8.62±0.92[bc]
48	9.01 ±0.45[ad]	10.75±0.73[ac]	9.66±0.92[bc]
72	12.09±1.22[ad]	17.01±1.41[ac]	12.08±0.82[bd]

Keys: *n = No of cows,*

a = significantly higher (P<0.05) along the column

b = significantly lower (P<0.05) along the column

c = significantly higher (P<0.05) across the row

d = significantly lower (P<0.05) across the row

Characteristics of proestrusoestradiol surge

The time to peak of serum proestrusoestradiol surges were significantly longer *P<0.05* in raining season followed by hot dry season (35.42 ± 4.25 hr) and then cold dry season (31.50 ± 5.41 hr) respectively, Table 5. The amplitude of proestrusoestradiol surges were significantly higher *P<0.05* in cold dry season (39.13 ± 5.27 pg/mL) followed by hot dry season (19.50 ± 2.52 pg/mL) and then raining season (17.63 ± 1.89 pg/mL). Duration of proestrus E_2 surges were significantly longer *p<0.05*in raining season (40.88 ± 7.10 hr) followed by hot dry season (35.50 ± 7.71 hr) and then cold dry season (24.25 ± 3.27 hr), respectively. Which corroborates the findings of (Wilson *et al.* 1998; Khan *et al.* 2020) who reported that plasma oestradiol concentration was reduced by heat stress in dairy cows. Also, (Roth *et al.* 2000) reported that reduction in the steroidogenic capacity of follicles under thermal stress is characterized by less aromatase activity of granulosa cells and decreased oestradiol concentration in the

dominant follicle. An effect that is consistent with decreased concentrations of LH and reduced dominance of the selected follicles (Roth *et al.* 2000). Potentially, adverse effects of low oestradiol production may lead to impaired oestrus duration and intensity; suppression of LH surge which, in turn, might impair events associated with ovulation; enhancement of the development of ovarian cysts; and alteration of corpus luteum development that affects progesterone production (Wilfenson *et al.* 2000). The time to peak and duration of proestrusoestradiol surge were increased by environmental heat stress, the reason for this increase was not clear.

Table 5: Characteristics of proestrusoestradiol surge of zebu cows in cold dry, hot dry and raining season

Season	*N*	Time to peak of E_2 surge (hr)	Amplitude of E_2 surge (pg/mL)	Duration of E_2 surge (hr)
Cold dry	12	31.50±5.41^{ad}	39.13±5.27^{ac}	24.25±3.27^{bd}
Hot dry	12	35.42±4.25^{ac}	19.50±2.52^{bd}	35.50±7.71^{ac}
Raining	12	52.25±7.08^{ac}	17.63±i.89^{bd}	40..88±7.10^{ac}

Keys: *n = No of cows*

a = significantly higher (P<0.05) along the row

b = significantly lower (P<0.05) along the row

c = significantly higher (P<0.05) along the column

d = significantly lower (P<0.05) along the column

Characteristics of pre ovulatory LH surge

The time to peak of serum pre-ovulatory LH surges were significantly earlier *P<0.05* in cold dry season (38.38 ± 2.29 hr), followed by hot dry season (44.40 ± 4.76 hr) and then raining season; (53.86 ± 5.37 hr), Table 6. The amplitude of pre-ovulatory LH surges were significantly higher *P<0.05* in raining season (11.8 ± 4.59 ng/mL), followed by hot dry season (4.18 ± 0.86 ng/mL) and then cold dry season (4.14 ± 0.3 ng/mL). There was no significant difference *P>0.05* between hot dry season (4.18±0.86 ng/mL) and cold dry (4.14 ± 0.3 ng/ml). Which corroborates the findings of (Gilad *et al.* 1993) who reported decrease in LH pulse amplitude during heat stress in heifers. The current finding however, disagreed with those of Rosenburg *et al.* (1982) who reported unchanged concentrations of pre-ovulatory LH surge in cows during heat stress. The reasons for these discrepancies are unclear, and it has been suggested by (Gilad *et al.* 1993) that these differences are related to pre-ovulatory oestradiol levels because the amplitude of tonic LH pulses and GnRH-induced pre-ovulatory plasma LH surge are decreased in cows with low plasma concentration of oestradiol, but not in cows with high plasma concentrations of oestradiol. Gilad *et al.* (1993), Lee (1993) have also reported that LH concentrations are decreased by heat stress and have drawn a conclusion that in summer, the dominant follicles develop in a low LH environment and these result in reduced oestradiol secretion from the dominant follicles leading to poor expression of oestrus, and hence, reduced fertility (Ahmed *et al.* 2015).

Duration of LH surges were significantly longer *P< 0.05* in cold dry season (18.63 ± 2.67 hr), followed by hot dry (11.80 ± 4.59 hr) and then raining season (8.71 ± 2.94 hr). There was no significant difference *P>0.05* between hot dry season (11.80 ± 4.59 hr) and raining season (8.71 ± 2.94 hr). Which is inconsistent the work of (Lemon *et al.* 1975; Scheams *et al.* 1977, Mandan and Johnson, 1973) who reported that the duration of LH surge of 15.3 hr in January in the Frisonne Francaise pie Naire breed of cow in temperate countries, also contradicts their findings in raining season that recorded longer duration of 21 hr against the 8.7 hr in the present study. The breed difference may explain the difference in the LH duration in the current study and other previous studies.

Table 6: Characteristics of pre ovulatory LH surge of zebu cows in cold dry, hot dry and raining season

Season	*n*	Time to peak of LH surge (hr)	Amplitude of LH surge (ng/mL)	Duration of LH surge (hr)
Cold dry	12	38.38±2.29^{ad}	4.14±0.3^{bd}	18.63±2.67^{ac}
Hot dry	12	44.40±4.76^{ac}	4.18±0.86^{bd}	11.80±4.59^{ad}
Raining	12	53.86±5.37^{ac}	11.80±4.59^{ac}	8.71±2.94b^{bd}

Keys: *n = No of cows*

a = significantly higher (P<0.05) along the row

b = significantly lower (P<0.05) along the row

c = significantly higher (P<0.05) along the column

d = significantly lower (P<0.05) along the column

Effect of Heat Stress on Oestrus Expression and number of Mounting

The results showed that oestrus duration was not significantly different *P*>0.05 among the study

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seasons cold dry season (2.2±0.2 days), hot dry season (2.2 ± 0.2 days) and raining season (2.0±0.0 days) respectively; Table 7. Ostrus intensity was significantly higher *P<0.05* in cold dry season (7.6 ± 0.93/hr) followed by raining season (6.2 ± 1.28/hr) and then hot dry season (4.4±0.81/hr) respectively. Which corroborate the reports of Ahmed *et al*. (2015), Pully *et al.* (2015) and Pennington *et al.* (1985), that mounting activity was reduced during hot weather when compared to cold weather leading to poor oestrus detection. Oestrus duration in the present study was the same in all seasons which is consistent with the report of Howel *et al.* (1994), but disagrees with the observation of (Gwadauskas *et al.* 1981 and Younas *et al.* 1993) who reported that heat stress reduced the duration of oestrus. We are associating this reduced mounting in heat stress season to the reduction of the proestrusoestradiol concentration that might have resulted to silence oestrus manifestation, because the cow may find it difficult to identify those cows in silence oestrus. The methods of heat detection (AM and PM role) used in the present study was not sufficient to capture all oestrus activities; some would have been missed out.

Table 7: Oestrus Duration and Number of Mounts of Zebu Cows during Cold Dry, Hot Dry and Raining Seasons

Season	Month	Oestrus duration (day)	Number of Mounts/day
Cold dry	4	2.2 ± 0.2^{bc}	7.6 ± 0.93^{ad}
Hot dry	3	2.2 ± 0.2^{bc}	4.4 ± 0.81^{bd}
Raining	5	2.0 ± 0.0^{bc}	6.2 ± 1.28^{ad}

Keys: *n = No of cows*

a = significantly higher (P<0.05) along the column

b = significantly lower (P<0.05) along the column

c = significantly lower (P<0.05) along the row

d = significantly higher (P<0.05) along the row

CONCLUSION

In conclusion, heat stress affect some reproductive indices of these cows by (i) increasing folliclesize without a corresponding increase inoestradiol concentrations, (ii) increasing or reducing pre-ovulatory LH surge concentration, (iii) increasing progesterone concentration and (iv) reducing mounting activity. However, it is uncertain whether these changes can be related to the well-documented low breeding efficiency during the hot dry months of the year in tropical environment.

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