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Research Article

Impact of Environmental Heat Stress on Ovarian Function of Zebu Cows

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Abstract | Study evaluated the impact of environmental heat stress on follicle diameter, and ovarian hormones concentrations. Twelve (*n*=12) non-pregnant, Zebu cows with 2-3 successive calving record were utilized. Temperature humidity index (THI) determined and rectal temperatures of cows were monitored. Cows were synchronized for oestrus using $PGF_2\alpha$. Follicular studies were carried out. Serum oestradiol and luteinizing hormones were monitored by Enzyme-Linked Immunosorbent Assay (ELISA). Blood samples for serum were collected for oestradiol and luteinizing hormones. Follicular diameters at 42 hr were longer (P<0.05) in hot dry season (8.088±0.52 mm) than in raining seasons (8.618±0.9 mm) and cold dry season (6.338± 0.68 mm). At 72 hr follicular diameters were longer (P<0.05) in hot dry season (17.006±1.41 mm) than cold dry (12.898±1.22 mm) and raining seasons (12.075±0.82 mm). Time to peak of oestradiol surges were shorter (P<0.05) in cold dry season (31.50±5.41 hr) than hot dry season (35.42±4.25 hr) and raining seasons (52.25±7.08 hr). Oestradiol concentrations were higher (P<0.05) in cold dry season (39.13±5.27 pg/dL) than hot dry (19.50±2.52 pg/dL) and raining seasons (17.63±1.89 pg/dL). Time to peak of LH surges were earlier (P<0.05) in cold dry season (38.38±2.29 hr) than hot dry season (44.40±4.76 hr) and raining season (53.86±5.37 hr). LH concentrations were higher (P<0.05) in raining season (11.8±4.59 ng/mL) than cold dry (4.14±0.3 ng/mL) and hot dry seasons (4.18±0.86 ng/mL). Duration of LH surges were longer (P<0.05) in cold dry season (18.63±2.67 ng/mL) than raining season (8.71±2.94 ng/mL). In conclusion, heat stress affected ovarian function by increasing follicles diameters, reducing oestradiol concentrations, and affected onset and duration of LH surges.

Keywords | Follicle, Hormone, Season, Zebu cows, Reproduction.

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INTRODUCTION

The high ambient temperature and solar radiation particularly during the hot dry season may influence the reproductive rhythm via the hypothalamic-Hypohpyseal-ovarian axis (Reddy and Rao, 2014). Cattle that are directly exposed to heat stress during hot season experience decrease fertility which is the problem faced by the farmers throughout the world (Wakayo et al., 2015). This persisting effect of heat stress on fertility of cow results in

changing the development of pre-ovulatory follicles during severe hot months (Roth et al., 2001a). Even minor alteration in the core body temperature were established to be sensitive enough to cause changes in the oestrous cyclicity in dairy cow (Fisher et al., 2008).

Heat stress has been reported to reduce plasma oestradiol level in dairy cows (Wilson et al., 1988, Wolfenson et al., 1997, Wolfenson et al., 1995) an effect that is consistent with decreased luteinizing hormones (LH) and reduced dominance of the selected follicle, however, this effect has not always been observed (Rosenberg et al., 1982). Decrease oestradiol concentration in the follicular fluid is more likely to occur after exposure to long-term, chronic (summer) heat stress than to acute heat stress (Roman-Ponce et al., 1981).

It has been suggested that heat stress may also act directly on the ovary to decrease its sensitivity to gonadotropin stimulation (Wolfenson et al., 1987). Also, the somatic cells within the follicle (theca and granulosa cells) can be damaged by heat stress (Roth et al., 2001a).

Since the main factors regulating ovarian activity are gonadotropin-releasing hormone from the hypothalamus and the gonodatropins, LH and FSH, from the anterior pituitary gland, some studies have investigated the impact of heat stress on the secretion of those hormones. The effects of heat stress on LH concentrations in peripheral blood circulation are inconsistent. Pennington et al. (1985); Gauthier (1986); Roman-Ponce et al. (1981) reported increased concentrations; while Roth and Wolfenson (2016); Khodei-Motlagh et al. (2011) reported decrease concentrations following heat stress. With regard to the pattern of LH secretion in heat stress cows, decrease in LH pulse amplitude has been reported by Gilad et al. (1993) and LH pulse frequency (Lee, 1993). The impact of heat stress on pre-ovulatory surge of LH is similarly controversial; a reduction of the endogenous LH surge by heat was reported in dairy cow (Roth and wolfenson, 2016). Studies by Ahmed et al. (2015) and Pully et al. (2015) have also shown decreased LH concentrations by heat stress. The studies concluded that hot season decrease LH concentrations, that decrease oestradiol secretion from the dominant follicles leading to poor oestrus expression, and hence, reduced fertility. It was howerver, hypothesised that there would be impact of environmental heat stress on the ovarian function of the indigenous cattle breed. To test this hypothesis, the objectives of the current study were to evaluate the effects of environmental heat stress on (i) follicle diameter, (ii) proestrus estradiol -17β concentration, time to peak and duration and (iii) preovulatory luteinizing hormone concentration, time to peak and duration.

MATERIALS AND METHODS

Study location

The study was conducted during the cold dry season (harmattan season) (November, 2017-February, 2018), hot dry season (March – May, 2018) and raining season (June – October, 2018) at the Veterinary Teaching Hospital, Research Station South Core, Federal University of Agriculture Makudi Benue State, Nigeria. Makurdi is situated in the southern Guinea savannah and located at latitude 7° 14′ North and longitude 8° 21′ East, and located at 600 meters above sea level. The area has a warm temperatures ranging from 24 to 40 °C, with relatively greater temperature occur between March and May (Time and Tor, 2016). The average rainfall is between 508 and 1016 mm annually. Three major annual seasons are experienced which are: the raining season (May-October) cold dry harmattan season (November-February) and the hot dry season (March-April) (Time and Tor, 2016) .

Determination of temperature humidity index (THI)

A year weather data for the period of this study was collected from the Air Force Base weather station located 2 kilometers from the research station. Collected data included average monthly air temperatures and relative humidity. The temperature humid index (THI) was calculated for all seasons using the formula developed by (Kbler, 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 in %. The determined THI values were used to identify heat stress seasons in Makurdi and to examine the seasonal variation of THI. The classification reported by (Du Preez et al., 1990) was adopted to quantify the intensity of heat stress.

Classification of environmental heat sress on cattle based 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, while THI values 75-86 was considered as heat stress.

Experimental animals and management

This research was carried out with the approval of the Ethical committee of the College of Veterinary Medicine, Federal University of Agriculture,Makurdi. Twelve (*n*=12) matured, cycling, non-pregnant, Zebu cows with 2-3 successive calving record, with average weight of 338.92±16.39 kg and body condition scores of 3.68±0.13 on a scale of 5, that were purchased in an open market were utilized over 3 replicate months (November, 2017-Febru-

ary, 2018, March-May, 2018 and June to October, 2018). The cows were identified with the use of large plastic ear tags and kept for 6 months period of stabilization, during which blood and fecal samples were 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 concentrate supplement provided 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 methods were used. This study was conducted between November, 2017 and October, 2018 (12 months) it was divided into three phases:

Phase I: November-February to represent cold dry season
Phase II: March – May to represent hot dry season
Phase III: June - October to represent raining season

Monitoring of rectal temperature

Rectal temperatures were monitored with the use of clinical thermometer at 12:00 -1:00 PM twice a week throughout the experimental period.

Oestrus synchronization

At the end of each phase cows were synchronized for oestrus using $PGF_2\alpha$ (Synchromate that contained Cloprostenol manufactured by Bremer Pharma Gmbh 34414 Warburg Germany Batch No. 26176) at 2 ml (500 µg) / cow intramuscularly. Two injections were administered 12 days apart.

Follicular study

Ultrasonic follicular studies, measuring the diameter of the lagest follicle of the first wave using a real time B-mode Ultrasound scanner (manufactured by Edan Instrument Inc. 1019// SkekoNashan Shenzhen 518067PR China with transrectal probe of 7.5 MHz linear array) was carried out transrectally daily for three days, beginning from 24 hr after administration of second dose of $PGF_2\alpha$ until ovulation occurred. Occurrence of ovulation was considered as the presence of ovulation depression fossa.

Sample collection

Blood sampling commenced 24 hr after administration of second dose of $PGF_2\alpha$. 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 2 hr for 72 hr for luteinizing hormone assay and 6 hr for 72 hr for oestradiol assay. Blood samples collected were kept at room temperature for 30 min and spun using centrifuge at 3000 rpm for 5 minute and serum samples were harvested and stored at -20 ^{0}C until analysis.

Serum Hormonal assay

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, Inc. Vantaa Findland). Assays were validated using the absorbance (OD) of the calibrators for each hormone according to the manufacturer instruction. 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 respectively.

The proestrus serum oestradiol (E_2) concentration obtained from the hormonal assay profiles were used to determine the serum proestrus E_2 surge; concentrations (peak E_2 magnitude), duration, (area under the E_2 secretion curve, and E_2 time to peak (area before the surge). A 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.

Serum pre-ovulatory luteinizing hormone (LH) concentrations obtained from the hormonal assay profile were used to determine the LH surge; peak LH magnitude (concentrations), area under the LH secretion curve (duration), and time to peak (area before the surge). Pre-ovulatory 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 2 ng/L following oestrus synchronization.

Statistucal analysis

Data 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

Temperature humidity index (THI)

Temperature humidity index (THI) values were 45 in cold dry season, 93.4 in hot dry season and 93 in raining season respectively (Table 1).

Table 1: Ambient Temperature, Relative Humidity and Temperature Humidity Index values for cold dry, hot dry and raining season

Season	Ambient Temperature (⁰C)	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 at cold dry, hot dry and raining seasons

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

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

Season	Duration of season (Months)	Temperature (˚C)
Cold dry	4	37.71 ± 0.04^{b}
Hot dry	3	38.07 ± 0.03^{a}
Rainy	5	38.06 ± 0.03^{a}

^{a,b} Means within a column labelled with different superscript letters differ significantly ($P<0.05$).

Follicular study

As shown in Table 3, 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). 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 72 hr 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.

Table 3: 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	Rainy Season *n*=12
24	6.34 ± 0.68^{cC}	8.09± 0.52^{abC}	8.62±0.92^{aB}
48	9.01 ±0.45^{cB}	10.75±0.73^{aB}	9.66±0.92^{abB}
72	12.09±1.22^{bA}	17.01±1.41^{aA}	12.08±0.82^{bA}

^{a,c} Means within a row labelled with different lower-case superscript letters differ significantly (P<0.05).
^{A,C} Means within a column labelled with different upper-case superscript letters differ significantly ($P<0.05$).
n=No of cows

Characteristics of proestrus oestradiol surge

The time to peak of serum proestrus oestradiol surges were significantly longer ($P<0.05$) in raining season (52.25±7.08) followed by hot dry season (35.42±4.25 hr) and then cold dry season (31.50±5.41 hr) respectively (Table 4). The amplitude of proestrus oestradiol surges were significantly higher ($P<0.0$) 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 (52.63±7.08 hr) (24.25±3.27 hr), respectively.

Table 4: Characteristics of proestrus oestradiol surge of zebu cows in cold dry, hot dry and raining season

Season	Month	Time to peak of E_2 surge (hr)	Amplitude of E_2 surge (pg/mL)	Duration of E_2 surge (hr)
Cold dry	4	31.50±5.41^{c}	39.13±5.27^{a}	24.25±3.27^{c}
Hot dry	3	35.42±4.25^{b}	19.50±2.52^{b}	35.50±7.71^{b}
Rainy	5	52.25±7.08^{a}	17.63±i.89^{bc}	40.88±7.10^{a}

^{a,c} Means within a column labelled with different lower-case superscript letters differ significantly ($P<0.05$).

Characteristics of pre ovulatory LH surge

As shown in Table 5, 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). The amplitude of pre-ovulatory LH surges were signifi-

cantly 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 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). 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).

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

Season	Month	Time to peak of LH surge (hr)	Amplitude of LH surge (ng/mL)	Duration of LH surge (hr)
Cold dry	4	38.38±2.29^{c}	4.14±0.3^{b}	18.63±2.67^{a}
Hot dry	3	44.40±4.76^{b}	4.18±0.86^{b}	11.80±4.59^{b}
Rainy	5	53.86±5.37^{a}	11.80±4.59^{a}	8.71±2.94b^{bc}

^{a,c} Means within a column labelled with different lower-case superscript letters differ significantly (P<0.05).

However, in cold dry season, time to peak of preovulatory luteininzing hormone surges were (38.38±2.29 hr) with aplititeude (4.14±0.3 ng/mL) and duration of (18.63±2.67 hr). Also the hot dry season recorded time to peak of preovulatory leutinizing hormone surges of (44.40±4.76 hr) with aplititude (4.18±0.86 ng/mL) and duration of (11.80±4.59 hr). And in the raining season time to peak of preonulatory leutinizing hormone surges were 53.86±5.37 hr), with aplititude of (11.8±4.59 ng/mL), and duration of (8.71±2.94 hr).

Ovulation rate

All cows ovulated within 78 -84 hr after administration of second dose of $PGF_2\alpha$,

DISCUSSION

Temperature humidity index (THI) in the present study was 45 in cold dry season indicaing that cows were under lower environmental heat stress, this finding was consistance with the finding of (Du preez, 2000) who reported that THI values <72 is associated with lower heat stress on cow. Also Johnson (1985) suggests that a THI value of 35 is asoociated with lower heat stress. The THI values of 93.4 in hot dry season and 93.0 in raining season indicated that cows were under environmental heat stress in hot dry and raining season, which corroborate the finding of (Du preez, 2000) who reported that cows are heat stress at THI values ranges from <73.

The rectal temperatures of cows in the present study ranged between 37.7 °C to 38.1 °C which contradicts the findings of Ma and Du (2010) who reported ranges between 38.0 °C to 39.0 °C in adult cows. This difference might arise due to the differences in climatic conditions and breed. The rise in rectal temperatures in hot dry season and raining season was directly proportional to rise in temperature humidity index that indicated critical environmental heat stress, this finding corroborates the finding of (Brown-Brand et al., 2003) who reported that amplitude of the body temperature rhythm increases as ambient temperature rises. But contradict the report of Berman et al. (1985) who noted that in man, dog and rat the rectal temperature was independent of air temperature but related to energy metabolism. The present study shows that high humidity index that is associated with environmental heat strss have increased the body temperature of Bunaji and Bokoloji cows in hot dry and raining season. This report corroborates the findings of (Correa-Calderon et al., 2004) who reported that ambient temperatures in excess of 25 °C have been shown to cause an increase in the body temperature of dairy cows, thereby increasing heat stress. However, the climatic condition and breed of cattle used in these studies may account for the differences.

The result of this study indicated that the folluicular diameters were longer in hot dry and raining season that are associated with severed environmental heat stress than in cold dry season that is associated with lower environmental heat stress, as evident by the presence of larger follicular size in hot dry season than raining and cold dry seasons. This finding showed that heat stress increases follicular size at early antral and pre-ovulatory stages of follicular growth. 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).

Serum oestradiol concentrations obtained in this study were decreased by environmental heat stress, which corroborates the findings of (Wilson et al., 1998) 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 (Wolfenson et al., 2000). The time to peak and duration of proestrus oestradiol surge were increased by environmental heat stress, the reason for this increase was not clear.

The results of the present study indicated that environmental heat stress increase time to peak of LH surge, the reason for this increase is not undertood. The amplitude of pre-ovulatory LH surges in this study was decreased by heat stress, which corroborates the findings of (Roth and Wolfenson, 2016) 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). LH concentrations are decreased by heat stress, conclusion have been drawn 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).

The result of this study demostrated that LH duration was 18.6 hr in January which corroborates the work of (Lemon et al., 1975, Scheams et al., 1977, Meidan 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, but 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.

CONCLUSION

In conclusion environmental heat stress have affected the reproductive axis of these cows by (i) increasing follicles diameter (ii) reducing proestrus oestradiol concentrations that is needed to trigger LH surge, (iii) increasing or reducing LH concentration that is needed to cause an ovulation, increasing time to peak of preovulatory LH surges and reduced it duration.

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CONFLICT OF INTEREST

The authors of this manuscript declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

NOVELTY STATEMENT

This research work was able to determine the proestrus oestradiol concentration that will be able to trigger a luteinizing hormone surge and luteinizing hormone concentration that can cause an ovulation in Nigerian zebu cow.

AUTHORS CONTRIBUTION

P. M. Dawuda designe the work and source for the funding, I. U. Ate Monitored the sample collection and proofread the draft, P. I. Rekwot edited the final draft and made contact for sample analysis, N. Wachida collected the sample analysed and wrote the draft of the manuscript.

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