RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v20i2.2

Ndumari *et al.*/*Sokoto Journal of Veterinary Sciences*, **20**(2): 87 - 94.

Impact of heat stress on follicular size, oestradiol concentration and oestrus expression in Nigerian Zebu cows

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Publication History:
Received: 10-08-2021
Revised: 14-09-2021
Accepted: 27-09-2021

Abstract

The study was conducted to evaluate the effect of heat stress on follicular size, oestradiol concentrations and oestrus expression in different seasons on Zebu cows. Twelve (n=12) matured, Zebu cows were utilized for over a year. The year was divided into 3 phases. Ambient temperature, relative humidity and rectal temperature were determined using. Cows were synchronized for oestrus. Ultrasonic follicular studies were carried out daily. Blood samples were collected after administration of a second dose of $PFG_2\alpha$ to assess serum concentration of oestradiol. The enzyme-linked immunosorbent assay technique was used to determine the concentration of oestradiol. Oestrus activities were monitored by visual observation. Follicle diameters at 24 hr were lower (P< 0.05) in the cold, dry season (6.34± 0.68 mm) than hot dry (8.09±0.52 mm) and the rainy season (8.62±0.9 mm). At 72 hr, follicular diameters were significantly higher P<0.05 in the hot, dry season (17.01±1.41 mm) than cold dry (12.90±1.22 mm) and the rainy season (12.08±0.82 mm). Time to peak of oestradiol concentrations was shorter P<0.05 in the cold, dry season (31.50±5.41 hr), followed by a hot, dry season (35.42±4.25 hr) and then rainy season (52.25±7.08 hr). The amplitude of oestradiol was higher P<0.05 in the cold, dry season (39.13±5.27 pg/mL) than hot dry (19.50±2.52 pg/mL) and rainy seasons (17.63±1.89 pg/mL). Durations of proestrus oestradiol surges were significantly higher P<0.05 in the rainy season (40.88±7.10 hr) followed by a hot, dry season (35.50±7.71 hr) and then cold dry season (24.25±3.27 hr.). The total number of mounting activities for two hr. was higher P<0.05 in the cold, dry season (7.6±0.93/hr) followed by rainy season (6.2±1.28/hr) and then hot dry season (4.4±0.81/hr). In conclusion, the study showed that heat stress affected oestrus expression by lowering the number of mounts per hour, increasing follicular size and reducing oestradiol concentration of these cows.

Keywords: Follicle, Estradiol, Heat stress, Season, Zebu cow

Introduction

Heat stress (HS) is a major challenge to livestock producers in many countries across the world, especially those located in the desert and tropical climates. As reported by the United States' Environmental Protection Agency (US EPA), the average global temperature is expected to increase by 0.3℃ to 4.8℃ by the year 2100, which may have a negative effect on animal production and efficiency if effective heat control strategies are not implemented (Wuebbles *et al.*, 2017). Harsh geographical climates and rising global temperatures make heat stress a critical concern for animal production (Wuebbles *et al.*, 2017). In tropical, subtropical and arid regions, high ambient temperature is the major factor jeopardizing animal production (Marai *et al.*, 2008; Hansen, 2009). 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 of cows exposed to heat stress results in the late manifestation of oestrus and a lengthened oestrus interval, leading to a high incidence of silent oestrus/anovulation and anoestrus in dairy cows (Piccione *et al.*, 2003).

Sexual behaviour peculiarities of Zebu breed animals, such as short length of oestrus and reduced mounting activity, can lead to errors in oestrus detection (Mattoni *et al.*, 1988). Zakari *et al.* (1981) observed that oestrus behaviour 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.* (1970) observed a high occurrence of long oestrous cycles and silent oestrus/anovulation 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. Reproductive studies in Nigerian indigenous Zebu cows by researchers (Dawuda *et al.*, 1987; Dawuda *et al.*, 1988; Pathiraja *et al.*, 1988 &Voh Jr *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 the opportunity to proffer solutions in solving the problem of infertility in these breeds of cattle. It was, therefore, hypothesized that there would be an effect of heat stress on the profiles of reproductive oestradiol, follicular size and oestrus expression in the indigenous Zebu breeds. Therefore, the objectives of the current study were to evaluate the effect of heat stress on proestrus oestradiol concentration, follicular size and, oestrus expression.

Materials and Methods

Study location

The study was conducted during the cold, dry harmattan season (November-February), hot, dry season (March – May) and the rainy season (June – October) 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 meters above sea level on latitude 7° 14′ North and longitude 8° 21′ East. The area has warm temperatures ranging from 24 to 40℃, with relatively greater temperatures occurring between March and May (Time & Tor, 2006). The average rainfall is between 508 and 1016 mm annually. Three major annual seasons are experienced which are: the rainy season (May-October), cold, dry season (November-February) and the hot, dry season, March-April (Time & Tor, 2006).

Determination of temperature-humidity index (THI)

A year weather data of the study period were collected from the Air Force Base Weather Station located 2 kilometres from the research station, using dry and wet bulb thermometer (minimum and maximum) and pitch evaporator barometer, respectively. Collected data included average, minimum and maximum monthly temperatures and relative humidity. The temperature-humidity index (THI) was calculated for all seasons using the formula developed by Kibler (1964). It is as follows:

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

Where: Ta = Mean Ambient Temperature in ℃; 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 environmental heat stress on cattle based on THI values

The 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

This research was carried out with the approval of the Ethical Committee of the College of Veterinary Medicine, Federal University of Agriculture Makurdi, Nigeria (FUAM/CVM/ETHICS/17/05). Twelve (n=12) matured, cycling, non-pregnant Zebu cows,

comprised of 6 Bunaji and 6 Bokoloji, age 4-5 years with an average weight between 350-450 kg and body condition scores between 4-4.5 on a scale of 5 that were purchased in open market were utilized over 3 replicate months (November-February, March-May and June - October). These two breeds were chosen because they are widely distributed across the country in high populations. 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 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 divided into three phases:
Phase I: November-February (cold dry season)
Phase II: March – May (hot dry season)
Phase III: June – October (rainy season)

Monitoring of rectal temperature: Rectal temperature was monitored with the use of clinical thermometer at 12:00 PM -1:00 PM throughout the experimental phases.

Oestrus synchronization: At the end of each phase, cows were synchronized for oestrus using $PGF_2\alpha$ (Synchromate ® that contained Cloprostenol manufactured by Bremer PharmaGmbh 34414 Warburg Germany Batch No. 26176) at 2500 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 2 hr 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 (Toshiaki *et al.*, 2014).

Follicular study: Ultrasonic follicular studies were conducted using a real-time B-mode Ultrasound scanner (manufactured by Edan Instrument Inc. 1019// SkekoNashan Shenzhen 518067PR China) with a transrectal probe of 7.5 MHz linear array. A transrectal scan was carried out once daily for three days, commencing 24 hr after administration of the second dose of $PGF_2\alpha$. Occurrence of ovulation was considered as the disappearance of the monitored follicle for two days consecutive scanning and disappearance of preovulatory follicle.

Blood Sample collection: Blood sample collection commenced 24 hr after administration of the 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 Ethylene diamine tetra acitic acid (EDTA) to harvest sera at every 6 hr for 72 hr for oestradiol 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 ^{o}C until analysis.

Serum Hormonal assay for oestradiol: Enzyme-Linked Immunosorbent Assay (ELISA) Kits (AccuBind, USA) and ELISA Reader (Thermo Scientific Multi task an EX (Vantaa Findland) were used according to manufacturer's instruction. The assay was validated, by reading the absorbance/optical density (OD) of the calibrator 0 pg/mL which was taken to be ≥1.3. The absorbance (OD) of the calibrator (0 pg/mL) of this assay was 2.563 which were>1.3, therefore, this assay was valid. Also, Inter- assay % Coefficient of Variation (CV) was 12.5 and intra-assay % CVs 5.6.

Determination of serum proestrus oestradiol (E2) surge: The 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 was the lowest elevated serum E2 value 24 hours after synchronized estrus. An E_2 surge was present irrespective of the peak values once it had exceeded 10 pg/mL following oestrus synchronization.

Statistical analysis

Data for rectal temperatures, follicular diameters, surge characteristics for proestrus oestradiol surges (time to peak, amplitude and duration) and oestrus expression were analyzed by repeated measure ANOVA using R Studio (R Core Team 2019). Turkey's Honesty Significant Difference Test was applied to determine the 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.

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

The result of this study shows that the rectal temperatures were significantly higher P<0.05; in hot dry season (38.07 ± 0.03℃) and the rainy season (38.06 ± 0.03℃) respectively than cold dry season (37.71 ± 0.04℃); Table 2. There was no significant difference P > 0.05 between hot dry (38.07 ± 0.03℃) and rainy seasons (38.06 ± 0.03).

Follicular dimensions

The follicular diameters at 24 hr after administration of the second dose of $PGF_2\alpha$ were significantly smaller P<0.05 in the cold dry season (6.34 ± 0.68 mm) than the hot dry season (8.09 ± 0.52 mm) and the rainy season (8.62 ± 0.9 mm), respectively; Table 3. There was no significant difference P>0.05 between hot dry (8.09 ± 0.52 mm) and the rainy season (8.62 ± 0.92 mm). At 48 hr follicular diameter did not differ among the different study seasons P>0.05 cold, dry season (9.01 ± 0.45 mm), hot, dry season (10.75 ± 0.73 mm) and rainy season (9.66 ± 0.92 mm) respectively. At 72 hr follicular diameters were significantly bigger P<0.05 in the hot dry season (17.01 ± 1.41 mm) than cold dry (12.90 ± 1.22 mm) and the rainy season (12.08 ± 0.82 mm), respectively. There was no significant difference P>0.05 between cold dry (12.10 ± 1.22 mm) and rainy seasons (12.08 ± 0.82 mm).

Characteristics of proestrus oestradiol surge

The time to peak of serum proestrus oestradiol surge was significantly shorter P<0.05 in cold dry season (31.50 ± 5.41 hr), followed by hot dry season (35.42 ± 4.25 hr) and then rainy season (52.25 ± 7.08 hr) respectively; Table 4. The amplitude of proestrus oestradiol surges were significantly higher P<0.00 in cold dry season (39.13 ± 5.27 pg/mL) than hot dry (19.50 ± 2.52 pg/mL) and rainy seasons (17.63 ± 1.89 pg/mL), respectively. There was no significant difference P>0.05 in the amplitude of proestrus oestradiol surges between hot dry (19.50 ± 2.52 pg/Ml) and rainy season (17.63 ± 1.89 pg/mL). Durations of proestrus oestradiol surges were significantly higher P<0.05 in the rainy 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.

Effect of Heat stress on oestrus expression

The results of this study showed that oestrus duration was not significantly different P>0.05 among the study seasons (2.2 ± 0.2 days), cold dry season (2.2 ± 0.2 days) hot dry season; (2.0±0.0days), rainy season respectively; Table 5. Oestrus intensity was significantly higher P<0.05 in the cold, dry season (7.6 ± 0.93/hr) followed by the rainy season (6.2 ± 1.28/**hr**) and then the hot dry season (4.4 ± 0.81/**hr**), respectively.

Table 1. Ambient temperature, relative humidity and temperature-humidity index values during cold dry, hot dry and rainy season

Season	Average Ambient Temperature (℃)	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

Table 2. Rectal temperatures of Zebu cows in cold dry, hot dry and rainy season

Season	Month	T ℃
Cold dry	3	37.71 ± 0.04^b
Hot dry	4	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

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^{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

Table 4. Characteristics of proestrus oestradiol surge of zebu cows in cold dry, hot dry and rainy 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}
Rainy	12	52.25 ± 7.08^{ac}	$17.63\pm$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

Table 5. Oestrus duration and number of mounts of zebu cows during cold dry, hot dry and rainy seasons

Season	Number of cows	Oestrus duration (hrs)	Number of Mounts/ hrs
Cold dry	12	2.2 ± 0.2^{bc}	7.6 ± 0.93^{ad}
Hot dry	12	2.2 ± 0.2^{bc}	4.4 ± 0.81^{bd}
Rainy	12	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

Discussion

The results of this study indicated that the diameters of the largest follicles at 24 hr were longer in the rainy season than in cold dry and hot dry season. Furthermore, at 72 hr after administration of the second dose of $PGF_2\alpha$ the pre ovulatory follicles were longer in the hot dry season than in cold dry and rainy seasons. This finding showed that the heat period increased the follicular diameter at early antral and pre-ovulatory stages of follicular growth, which is inconsistent with the observation of (Rosenberg *et al.*, 1977) who reported that heat stress decreased the diameter of follicles. At 72 hr after administration of the second dose of $PGF_2\alpha$ (i.e. during the late antral stage of follicular development), the diameter of the largest follicles increased uniformly in all seasons and this is probably because the animals physiologic homeostasis had adjusted to the heat stress condition. Follicular size did not indicate the functionality of the dominant follicle in the present study because follicular size was larger in hot dry season whereas oestradiol concentration was higher in cold dry season which is consistent with Shehab-El-Deen *et al.* (2010) who reported that follicular size is not a good indicator of functional follicular dominance.

Serum oestradiol concentrations obtained in this study corroborate the reports of (Wolfenson *et al.*, 1997; Khan *et al.*, 2020) that a reduction in the steroidogenic capacity of follicles under thermal stress which is characterized by less aromatase activity of granulosa cells and decreased oestradiol concentration in the dominant follicle. Also, Wilson *et al.* (1998) and Wolfenson *et al.* (1994) have reported that plasma oestradiol concentration was reduced by heat stress in dairy cows; an effect that is consistent

with decreased concentrations of LH and reduced dominance of the selected follicles. However, the results of the current study in raining season disagreed with the reports of Wilson *et al.* (1998) and Wolfenson *et al.* (1995). This difference could probably be because raining season in Makurdi Benue Sate Nigeria is characterized by high ambient temperatures and humidity at the early part of the season especially in May to June (Time & Tor, 2006). Potentially, adverse effects of low oestradiol production may lead to impaired oestrus expression, duration and intensity; suppression of LH surge which, in turn, might impair events associated with ovulation; development of ovarian cysts; and alteration of corpus luteum function. This may lead to reduced progesterone production (Wolfenson *et al.*, 1994).

The findings of this research show that cows mounted more frequently when on standing oestrus in cold dry season than in raining and hot dry seasons, which corroborate the report of Pennington *et al.* (1985), Ahmed *et al.* (2015) and Pully *et al.* (2015) 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 *et al.* (1994), but in disagreement with the report Howell of others (Gwazdauskas *et al.*, 1981; Younas *et al.*, 1993) that heat stress reduced the duration of 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.

In conclusion, heat stress has affected oestrus expression of these cows by (i) reducing mounting activity, (ii) reducing oestradiol concentrations and increasing follicle size

Acknowledgement

We gratefully acknowledge the Tertiary Education Trust Fund (TETFund) Nigeria for its financial support we received to execute this project. We also acknowledge the support of the Federal University of Agriculture, Makurdi Benue State Nigeria for providing facilities for us to carry out this research work to a successful conclusion. We thank The National Veterinary Research Institute, Vom Nigeria for allowing us to carry out the Enzyme Linked Immunosorbent Assay technique in its laboratory. We are grateful to Dr U. Husaini, Dr Y. S. Wungak and Dr A. Ebuji, for their technical assistance during the Laboratory work. We appreciate Dr S. Ode and Dr V. Ahur for helping us with the statistical analysis.

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.

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