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Original Article

Characterizing the ovarian follicular dynamics and ovarian hormonal profile of Lakhimi cow during the estrus cycle

Anupam Datta[1], Anubha Baruah[2], Arundhati Bora[2], D. J. Dutta[2], J. Goswami[2], D. Bhuyan[2], and Sukanta Das[3*]

[1] *Animal Resources Development Department, Government of Tripura, Kunjaban, Tripura, 799006 India*

[2] *Faculty of Veterinary Science, Khanapara Assam Agricultural University, Khanapara, Guwahati, 781022 India*

[3] *College of Veterinary Sciences and Animal Husbandry, R. K. Nagar, West Tripura, Tripura, 799008 India*

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Abstract

Lakhimi cattle is a promising indigenous dual breed in Assam and adjoining tropical area of Northeastern India, with valuable traits. This study aimed to monitor ovarian follicular dynamics and hormonal profiles during the estrus cycle in Lakhimi cows. The predominant one of two-wave follicular cycles (66.7%) was observed in Lakhimi cows, with a significantly shorter duration of the ovulatory wave compared to three-wave cycles ($p<0.05$). Two-wave cycles exhibited a higher antral follicle count (4.33 ± 0.49 nos) and smaller dominant follicles (11.51 ± 0.54 mm) compared to three-wave cycles. Early luteal regression was observed in two-wave cycles (14.15 ± 1.50 days). Hormonal dynamics revealed the peak progesterone concentration on 10th day (10.94±1.93 ng/ml), estradiol on the 20th day, and the day of heat and luteinizing hormone (LH) was the 1st day. These insights about Lakhimi cow's reproductive physiology can aid in breeding, conserving, and promoting this indigenous breed.

Keywords: estrus cycle, follicular wave, indigenous cow, Lakhimi cow, ovarian hormone

1. Introduction

The indigenous cattle of Northeastern India is well known for its high endurance, resistance, adaptability, and heritability. Lakhimi is one of the most popular indigenous cattle breeds in Assam and other nearby northeastern states of India, constituting 7.9 million out of the 8.4 million heads of indigenous cattle (Directorate of Animal Husbandry and Veterinary Department, Assam, India). Successful reproduction is an indispensable phenomenon that helps uplift cattle husbandry as well as preserve the germplasm with valuable traits. Deviations in reproductive rhythm can cause issues like low conception, poor performance, and infertility. Ovarian function is characterized by a follicular wave associated with an interplay of hormones, genes, environment, nutrition, breed, and individuality. Understanding the ovarian-follicular interplay during the estrus cycle is essential to interpreting the reproductive behavior of this breed (Dodiyar, Brar, Singh, & Honparkhe, 2022; Kim, 2018; Minela, Gibb, & McBeth, 2023; Muraya, Mutembei, & Mutiga, 2015). The study of ovarian follicular dynamics with characteristic wave numbers, growth, and regression of follicles during the estrous cycle is not a new venture (Barkawi *et al*., 2009; Dodiyar *et al*., 2022; Fortune, 2003; Kim, 2018; Minela *et al*., 2023; Muraya *et al*., 2015). Variability in follicular wave numbers has been recorded across different breeds (Savio *et al*., 1993; Sirois & Fortune, 1988). The association between ovarian follicular number in bovines has with high repeatability been

*Corresponding author
Email address: sukanta.23@gmail.com

reported by many authors (Morotti, 2017; Seneda, 2019) and reproductive performance is reported by Lima *et al*. (2020); Moraes, Morotti, Costa, Lunardelli, and Seneda, (2019); Morotti (2018, 2019). The count of antral follicles was reported to be associated with reproductive performance (Ireland, 2008; Mossa, 2012; Singh, Dominguez, Jaiswal, & Adams, 2004). Variations in the ovarian follicular dynamics were recorded amongst the classes Bos indicus and Bos taurus (Dodiyar *et al*., 2022) in timing, cycle patterns, follicular number, size, duration, and individuality (Figueiredo, Barros, Pinheiro, & Soler, 1997; Moraes *et al*., 2019; Morotti, 2018; Seneda, 2019). So far, no study has reported on follicular dynamics in Assam local cattle (Lakhimi). There is evidence of research towards applications of data on ovarian follicular dynamics in assisted reproductive technologies (Bizarro-Silva *et al*., 2018; Hatzirodos *et al*., 2014; Rossetto *et al*., 2016; Silva, Hurk, & Figueiredo, 2016). An understanding of follicular dynamics is required for scheming effective estrous synchronization and ovulations, which would help in conservation and improve the fertility level and propagation of this breed.

2. Materials and Methods

2.1 Ethical permission

The experiment was approved by the Institutional Ethical Committee Approval 770/ac/CPCSEA/FVSc/AAU/ IAEC/17-18/569.

2.2 Experimental animals and their management

Ten (10) Assam local (Lakhimi) adult, non-pregnant, healthy regular cyclic cows (non lactating) with good body condition were selected for the study (Figure 1). They were housed in well-ventilated hygienic sheds and maintained under the same conditions throughout the study and provided with *ad libitum* fresh and clean drinking water, feed, and fodder. The study was conducted at the Experimental Animal Shed, Department of Veterinary Physiology.

2.3 Preparation of animals for ultrasonography and scanning of ovary

The animal selected for ultrasonography was restrained in a standing position for scanning in the Travis. All cows were scanned using a real-time, B-mode ultrasound scanner (M turbo C, Fujifilm Sonosite Inc., Bothell, USA) equipped with a 7.5–9.0 MHz linear rectal transducer adapted for transrectal examination in large domestic animals as per the procedure mentioned by Tom, Pierson, and Adams, (1984) in cows. The ovaries of each cow were examined every day through an estrous cycle, starting from the observed onset of estrus (day 0) to the subsequent standing estrus. The day-to-day identity of follicles was profiled in all the cycles based on which the parameters of follicular development were arrived at, as described by Tom *et al*. (1998). The number of follicles recruited (as evidenced by small 3–4 mm-sized follicles on the day of emergence) and the characteristics of the dominant follicles (DFs) during the follicular wave and ovulatory wave were compared. As in the follicular study, the luteal structure was mapped during each examination, and the developmental parameters, viz., the maximum diameter, the day at which it attained the maximum diameter, the day of initiation of constant regression, the position of the antral follicle, and the luteal dimensions, were recorded in different waves. Each antral follicle (≥ 3mm in diameter) in each section was drawn on an ovarian map. Small, medium, large, and ovulatory follicles (Figure 1) were identified with diameters of 3-6 mm, 6-9 mm, and above 9 mm, respectively (Tom *et al*., 1998).

Figure 1. Photograph of a female cyclic Lakhimi cow in cattle of Assam

2.4 Blood samples and hormone assay

Blood samples (5 mL) were collected on every alternate day of an estrous cycle in heparinized tubes and placed on ice immediately after collection. The serum was separated, transferred to cryovials, and stored at -20˚C until assayed for hormones. The concentration of progesterone was measured with a solid-phase RIA kit (Progesterone C.T. RIA kit (Pkg: 100 T) Batch No. 181008D, M/s Beckman Coulter), and the radioactivity was counted in a 125I (STRATEC Germany) gamma counter. Estradiol and LH were estimated based on competitive enzyme immunoassay technique using Bovine Estradiol, Catalog No-CSB-E08173b (CUSABIO) andserum LH (catalog no CSB-E12826B (CUSABIO) ELISA kit. Analysis of results was done by using StepOne software v 2.1. The levels of various hormones were analyzed by Independent Sample T-test using SPSS (Statistical Package for Social Sciences) and Graph-pad Prism V-4.0 software. $P < 0.05$ was considered statistically significant.

3. Results and Discussion

Follicular wave cycles in Lakhimi cows in general with the predominance of two follicular waves, 7 out of 10 cattle ($p > 0.05$), was similar to the observations recorded by various authors (Dodiyar *et al*., 2022 in Sahiwal cow; Gaur & Purohit, 2007 in Rathi cows; Islam *et al*., 2020 in Red Chittagong cows). However, cycles with three (Gambini, Moreira, Castilho, & Barros, 1998), four or five follicular waves (Viana, Fereirs, de, Camargo, & De, 2000) were also reported in indigenous cows. The predominance of three follicular wave cycles had been reported in Bos Indicus (Dodiyar *et al*., 2022 in Sahiwal, Mollo *et al*., 2007 in Nelore, Viana *et al*., 2000 in Brahman and Gir cows). The predominance of two-wave follicular cycle was reported in Bos Taurus by Sartori, Monteiro and Wiltbank, (2016);

Townson *et al*. (2002). However, factors like the length of the luteal phase (Figueiredo *et al*., 1997), follicular size, estradiol concentration, peaks of Follicle Stimulating Hormone (Adams, Jaiswal, Singh, & Malhi, 2008), and insulin-like growth factors may have important roles in determining waves of the follicle during the estrus cycle.

In the present study, the emergence of the first and second waves (on 1.16 ± 0.30 and 10.83 ± 0.47 days respectively) and on 0.83 ± 0.16, 7.33 ± 0.49 and 12.16 ± 0.47 days during two and three wave cycle (Figure 2a, 2b) was followed by selection (divergence) of DF (Tables 1 and 2) and can be compared with those of Bahaman heifers (Rhodes, Fitzpatrick, Entwistle, & De'ath, 1995) and with Nelore cattle (Figueiredo *et al*., 1997), Viana *et al*. (2000) for Gir cows, and Muraya *et al*. (2015) for the Kenyan Boran, respectively. However, Islam *et al*. (2020) reported 2.00 ± 1.50 and 12.00 ± 2.10 days respectively in Red Chittagong cows. In line, Gaur and Purohit (2007) reported the emergence of the first follicular wave on day 2.10±0.36 and 0.7±0.5 after ovulation in the Rathi cows in 2 and 3-wave cycles, respectively. The emergence of the second wave can be comparable with Sahiwal cows reported earlier for results on 3-wave compared to the 2-wave estrous cycle (Dodiyar *et al*., 2022).

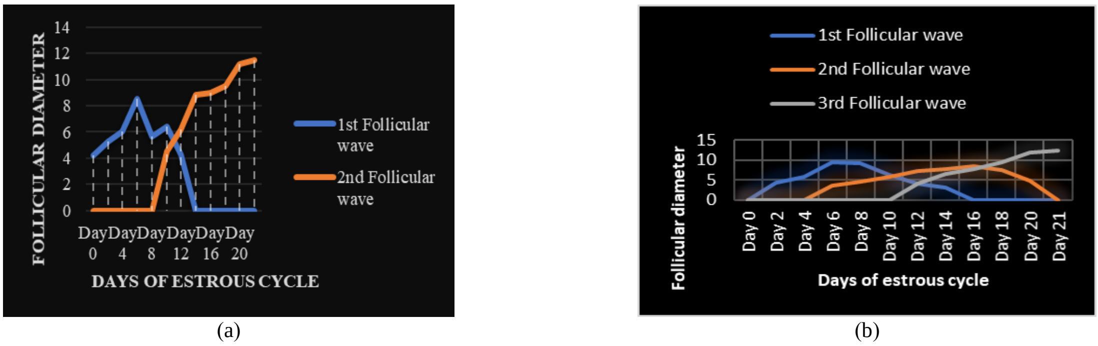

Figure 2. Follicular dynamics of Lakhimi cow with 2 waves (a) showing blue line indicating the 1st wave and red line as ovulatory wave, and three (b) follicular waves indicated by blue (1st), red (2nd) and green (ovulatory wave), during estrous cycle

Table 1. Ovarian follicular characteristics (Mean±SE) of two wave cycle in Lakhimi cows

Characteristic	Follicular wave	
	1st (Anovulatory)	2nd (Ovulatory)
Day of Wave emergence	1.16±0.30	10.83±0.47
No. of Follicles (>3mm)	3.5±0.42	4.33±0.49 *
Day of emergence of DF	5.5±0.42	14.83±0.60
Maximum diameter of DF	8.49±0.49	11.51±0.54 *
Day of maximum diameter	6.83±0.40	20.16±0.30
Day of onset of atresia	8.83±0.30 *	-
Day of termination of wave	13.66±0.42	-
Duration of persistence of wave (days)	14.33	10.66±0.49

Level of signifcance (P > 0.05) is indicated by superscript *

Table 2. The follicle diameter (mean±SE mm) at different days in two wave cycle in Lakhimi cows

Day of estrous cycle	1st Follicular wave	2nd Follicular wave
Day 0	4.2000±0.44721	-
Day 2	5.3000±0.49193	-
Day 4	6.0117±0.48666	-
Day 6	8.4967±0.49165	-
Day 8	5.6950±0.53762	-
Day 10	6.3983 ± 0.59200	4.4983 ± 0.63518
Day 12	4.3117 ± 0.60159	6.1950 ± 0.40261
Day 14	-	8.8100 ± 0.52472
Day 16	-	8.9950 ± 0.53611
Day 18	-	9.4950 ± 0.58092
Day 20	-	11.2200 ± 0.68172
Day 21	-	11.5117 ± 0.54980

Level of signifcance (P > 0.05) is indicated by superscript *

The day of dominant follicle selection in the present study during two and three waves was delayed compared to Sahiwal cows. However, the average diameter of the dominant follicle (8.49±0.49 mm,11.51±0.54 mm in two wave and 9.41 ± 0.87 mm,8.51 ± 0.26 mm, 12.41 ± 0.69 mm in three wave) in the Lakhimi cow was higher compared to the Sahiwal cow (Tables 1 and 3) (Dodiyar *et al*., 2022). This is in agreement with the observations in Kenyan Boran cows (Muraya *et al*., 2015). However, in Bos Taurus breeds, follicular selection occurs when the largest developing follicle reaches 8.5 to 9.0 mm in diameter (Ginther, Kot, Kulick, Martin, & Wiltbank, 1996; Sartori, Haughian, Shaver, Rosa, & Wiltbank, 2004) whereas, in the case of ovulatory wave the diameter at the time of selection was between 8.3 to 9.8 mm in Holstein cattle (Bastos *et al*., 2010). So, it is evident here from the literature that the diameter of the follicle at selection differs in Bos Indicus and Bos Taurus. In contrast, Sartori *et al*. (2016) observed a similarity between Bos Taurus and Bos Indicus (Nelore) in regard to follicular deviation, which could be due to a difference in follicular growth rate (Sartori, Fricke, Ferreira, Ginther, & Wiltbank, 2001). The maximum size of the dominant follicle (DF) of the first and second follicular waves (Tables 2 and 4) was smaller (8.49 ± 0.49 and 11.51 ± 0.54 mm) than in Red Chittagong cows (10-11 mm) (Islam *et al*., 2020), Holstein heifers (14 - 20 mm), and Brahman heifers (13 - 18 mm) (Rhodes *et al*., 1995) but was similar to the DF (8 - 9 mm) reported for Chinese Yellow Cattle (Vasconcelos, Sartori, Oliveira, Guenther, &Wiltbank, 2001). However, the maximum diameter of DF during three follicular waves in Lakhimi cows was in agreement with the observations made by Gambini *et al*. (1998) in Gir and Nellore cows, Gaur & Purohit, (2007) in Rathi cows and Filho, Olivera, Caldaa, & Lima, (2001) in crossbred cows. Though the pattern of growth and turnover of DF were similar to Nellore, and Brahman cows, the sizes of DF in the ovaries of Lakhimi cows can be compared with Red Chittagong Cow but are smaller than those of Nelore and Brahman heifers and Holstein.

The maximum size of the DF of the first and second follicular wave in two wave estrous cycles was 8.49 ± 0.49 and 11.51 ± 0.54 mm on mean days 6.83 ± 0.40 and 20.16 ± 0.30 ($p<0.05$) while the maximum size of the DF of the first, second and third follicular wave in three wave cycle was 9.41 ± 0.87, 8.51 ± 0.26 and 12.41 ± 0.69 mm on the mean days at 6.16 ± 0.47, 15.5 ± 0.56 and 20.33 ± 0.33 in Lakhimi cows (Tables 2 and 4). This finding matches the reports on Rathi cows by Gaur & Purohit, (2007); Zebu cows by Viana *et al*. (2000) and crossbred cows by Filho, Olivera, Caldaa, and Lima, (2001). The difference in attaining a maximum diameter of DF may be because the second wave emerges during the period of higher progesterone production by the CL, whereas the first and third waves emerge respectively during the luteogenic and luteolytic periods (Gambini *et al*., 1998; Viana *et al*., 2000). During the first follicular wave due to low concentration of progesterone, satisfactory negative feedback cannot be generated on the hypothalamic-pituitary to prevent the release of luteinizing hormone. The second wave started in the presence of fully formed CL that secretes a high concentration of progesterone, sufficient to inhibit the release

Table 3. Ovarian follicular characteristics (mean±SE mm) of three wave cycle in Lakhimi cows

Characteristic	Follicular wave		
	1st (Anovulatory)	2nd (Anovulatory)	3rd (Ovulatory)
Day of Wave emergence	0.83 ± 0.16	7.33 ± 0.49	12.16 ± 0.47
No. Of Follicles (>3mm)	3.83 ± 0.47	2.83 ± 0.30	3.66 ± 0.33
Day of emergence of DF	4.83 ± 0.30	9.16 ± 0.31	15.5 ±0.5
Maximum diameter of DF	9.41 ± 0.87	8.51 ± 0.26	12.41 ± 0.69*
Day of maximum diameter	6.16 ± 0.47	15.5 ± 0.56	20.33 ± 0.33*
Day of onset of atresia	7.83 ± 0.30	15.16 ± 0.40*	
Day of termination of wave	14.33 ± 0.42	19.16 ± 0.47	
Duration of persistence of wave (days)	14.16 ± 0.40	11.83 ± 0.60	7.16 ± 0.47

Level of signifcance ($P > 0.05$) is indicated by superscript *

Table 4. The follicle diameter (mean±SE mm) at different days in three wave cycle

Day of estrous cycle	1st Follicular wave	2nd Follicular wave	3rd Follicular wave
Day 0	-	-	-
Day 2	4.3117 ± 0.43593		
Day 4	5.8450 ± 0.91365		
Day 6	9.4117 ± 0.87254	3.5067 ± 0.47532	-
Day 8	9.2283 ± 0.54345	4.6283 ± 0.49127	-
Day 10	6.2317 ± 0.54287	5.7117 ± 0.56724	-
Day 12	4.1450 ± 0.55115	7.3283 ± 0.60960	4.0483 ± 0.46914
Day 14	3.0083 ± 0.70524	7.7300 ± 0.38221	6.5283 ± 0.77125
Day 16	-	8.5167 ± 0.26594	7.8133 ± 0.78803
Day 18	-	7.5267 ± 0.82291	9.3967 ± 0.58485
Day 20	-	4.8133 ± 0.44034	11.8017 ± 0.59335
Day 21	-	-	12.4150 ± 0.69786

Level of signifcance ($P > 0.05$) is indicated by superscript *

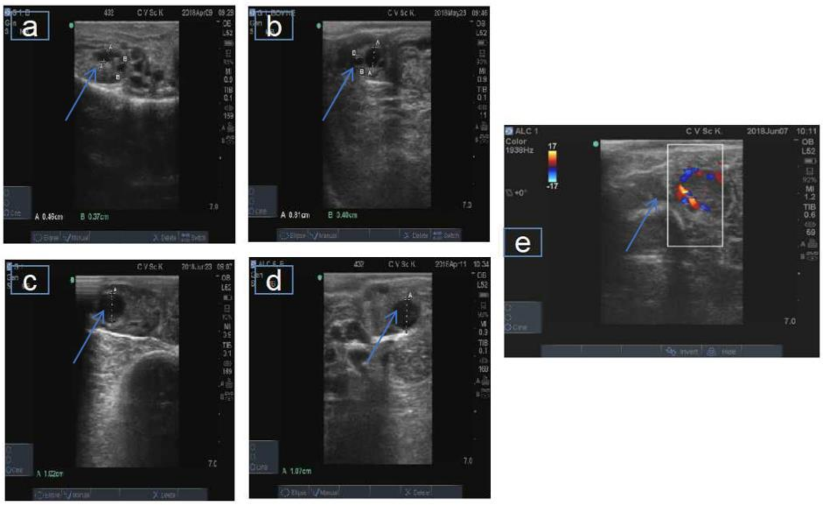

Figure 3. Ultrasound images showing (arrow) follicular development and ovarian changes during estrous cycle in Lakhimi cows: (a) small follicles, (b) medium, (c) large, and (d) ovulatory follicle. (e) Corpus luteum with blood vessels

Table 5. Serum progesterone, estradiol, and LH (ng/ml) profiles (mean±SE) in Lakhimi cows

Day of estrous cycle	Mean concentration of serum progesterone (ng/ml)	Mean concentration of serum estradiol (ng/ml)	Mean concentration of serum LH (ng/ml)
Day 0	0.41±0.09	192±11.35 *	2.61±0.53
Day 1	0.43±0.08	29±3.03	7.97±1.03 *
Day 2	0.56±0.08	28±2.79	1.85±0.41
Day 4	4.63±0.54	25±0.44	1.82±0.41
Day 6	7.69±1.43	20±1.6	0.91±0.20
Day 8	8.92±1.43	25±1.4	0.91±0.20
Day 10	10.94±1.93*	21±1.2	0.79±0.09
Day 12	10.34±0.93*	15±1.58	0.67±0.09
Day 14	7.77±1.07	12±1.24	0.54±0.09
Day 16	3.52±0.67	12±1.44	0.78±0.12
Day 18	1.54±0.40	79±9.18 *	1.01±0.12
Day 20	0.71±0.14	118±13.81*	1.52±0.14

Level of signifcance (P > 0.05) is indicated by superscript *

of LH. Low-frequency pulses of LH were observed during the luteogenic and luteal phases of the estrous cycle, leading to regression of the DF and initiation of a new follicular wave (Gambini *et al*., 1998). The third wave coincides with the reduced secretion of progesterone by the CL, increasing LH to a peak. This phase is to check the rapid follicular growth and ovulation (Savio *et al*., 1993). The lower concentration of progesterone present during luteogenic and luteolytic periods may be associated with higher gonadotrophic concentration and consequently with higher diameter and persistency of DFs. The maximum size of the wave DF is proportional to the FSH release inhibition (Ginther *et al*., 1996) but the final maturation and ovulation of DF are dependent on a high frequency of LH pulses (Roche & Boland, 1991). Nutritional status also influences the follicular dynamics during the estrous cycle. Maximum diameter and persistence of DF are reduced in case of a negative energy balance (Rhodes *et al*., 1995).

The length of the estrous cycle was minimally shorter (20.16±0.30 days) than for the 3-wave estrous cycle (Tables 1 and 3). However shorter mean length of the wave estrous cycle of more than two days compared to the 3 wave estrous cycle was reported by Dodiyar *et al*. (2022); Noseir *et al*. (2003) in Sahiwal cows. Bu, Adams, Nasser, Pierson, and Mapletoft (2003) reported that the wave patterns were influenced by parity in Nelore cattle, nutritional supplements, and heat stress.

In two-wave cycles, ovulation occurs on the mean (± SE) day 20.16 ± 0.30 whereas in three wave cycle, ovulation occurred on the mean (± SE) day 20.33 ± 0.33. The days of wave persistence were shorter in three wave compare to two wave cases (Tables 1, 3, and 4). Follicular wave lengths during 1st and 2nd waves in Lakhimi cows were delayed compared to Red Chittagong cows (Islam *et al*., 2020) on 12.44 ± 0.08 and 8.07 ± 0.06 days, but concomitant to Nelore cattle on day 14.75 ± 0.70 and 9.05 ± 0.69 (Figueiredo *et al*., 1997), respectively. The three wave cycles in Lakhimi cows were similar to Zebu cows (Viana *et al*., 2000) and crossbred cows (Filho *et al*., 2001). The variations in the persistence of waves in different breeds may be influenced by the pulsatile frequency of LH (Lucy *et al*., 1992), and progesterone (Gambini *et al*., 1998). In the present study the day of emergence, followed by selection (divergence) of DF in Lakhimi cows were correspondent to observations by

Figueiredo *et al*., (1997) in Nellore cows and in Gir cows by Gambini *et al*. (1998).

In two wave estrous cycle, 3.5 ± 0.42 and 4.33 ± 0.49 counts of follicles (>3 mm) emerged at the first and second wave ($p<0.05$) while in three wave cycles 3.83 ± 0.47, 2.83 ± 0.30 and 3.66 ± 0.33 counts of follicles (>3 mm) emerged at first, second and third wave in Lakhimi cows (Tables 1 and 3) matching the Alvarez *et al*. (2000) in Brahman cows, Amrozi, Kamimura, Ando, & Hamana, (2004) in Holstein cows, and Dodiyar *et al*. (2022) in Sahiwal. However, Islam *et al*. (2020) reported 8.00 ± 1.50 and 6.50 ± 1.70 follicles in Red Chittagong Cows. Variations in the number of follicles reported may be due to individual differences (Boni, Roelofsen, Pieterse, Kogut & Kruip, 1997), serum growth hormone, and IGF-I (Lucy *et al*., 1992) concentration, season (Lammoglia *et al*., 1998) and nutrition status (Lucy *et al*., 1992) of cow. The ovarian antral follicular count is an important indicator of fertility and the number of follicles varies with breed, ovarian dimension, heritability, and size of the dominant follicle (Lima *et al*., 2020). The mean day of follicular atresia in Lakhimi cows agreed with the findings by Ginther *et al.* (1996) in Holstein cows, and Gaur and Purohit (2007) in Rathi cows. The day of ovulation accounted in the present study for two and three waves agrees with Gambini *et al*. (1998) in Gir and Nelore cows, and Pinheiro *et al*. (1998) in European cows. Time of ovulation may be variable because these studies were done daily, so there may be chances of biases in the detection time of the start of overt signs of estrus.

It was observed that the maximum Corpus luteal (CL) diameter was 17.54 ± 0.67 mm for two follicular wave cycles and 18.25 ± 1.32 mm for three waves ($p<0.05$). Luteal regression took place at 14.15 ± 1.50 and 16.00 ± 1.50 days of the estrus cycle, respectively ($p<0.05$). The growth profile of the CL was similar between 2-wave versus 3-wave patterns until the onset of regression, and similar observations were noted by (Jaiswal, Singh, Marshall, & Adams, 2009). The duration of growth of ovulatory follicle in Lakhimi cows can be comparable with two follicular wave cycles of Red Chittagong cows (8.24 ± 0.56 and 9.51 ± 0.68 days) Islam *et al*. (2020), Nelore cattle with two and three follicular wave cycles (8.65 ± 0.73 and 7.0 ± 0.51 days) Figueiredo *et al*. (1997), and Holstein heifers with two and three follicular waves cycles (7.50 ± 0.19 and 5.90 ± 0.30 days). Slower rates of follicular growth were recorded compared to those of Nelore cattle and Holstein heifers, which may be a breed variation. The follicular deviation after ovulation in Lakhimi cows in the present study during the two and three-wave cycle was approximately 2 days and Islam *et al*. (2020) reported 1.53 days in Red Chittagong cows. The emergence of the first follicular wave does not usually occur until 24 h after ovulation in Lakhimi cow; therefore, follicular deviation seems to occur earlier than in Nelore and Holstein females (Fortune, 2003).

The hormonal dynamics in Lakhimi cows revealed that peak progesterone concentration occurred on the 10^{th} day (10.94±1.93 ng/ml), estradiol peaked on the 20th day (the day before estrus), and luteinizing hormone (LH) reached its highest levels on the 1^{st} day of estrus, followed by a subsequent decline in concentration. The serum concentration dynamics of hormones along the estrus cycle in Lakhimi cows were similar as reported by Islam *et al*. (2020); Saini, Yadav, Kumar, and Pandey (2021) in indigenous cows. Islam *et al*. (2020) observed a progressive increase in serum progesterone from <1 ng/ml to ≥27 ng/ml on days 4 - 6 and consequent follicular deviation in Red Chittagong cows. The serum concentration trend of estradiol in the Lakhimi cow during the estrous cycle agreed with Islam *et al*. (2020). Only a peak concentration of LH in the Lakhimi cow on day 1 of the estrous cycle, was then maintained at a very low level throughout the cycle which is crucial for final oocyte maturation as reported by Dias *et al*. (2010). However, the sizes of the dominant follicle and pre-ovulatory follicles were smaller than for the cross-breed Holstein and can be comparable with the local indigenous breeds like zebu, red Chittagong cows, Bhraman cow, etc. Lima *et al*. (2020) have pointed out the close relationship between follicular dynamics, progesterone concentrations, and pregnancy rates with a high or low antral follicle count in Bos Indicus.

4. Conclusions

The ovarian follicular waves in Lakhimi cows are predominantly characterized by two-wave cycles, featuring a lower antral follicular count, smaller dominant follicles, and reduced concentrations of LH, progesterone, and estradiol compared to three-wave cycles. These findings provide valuable insights into the reproductive physiology of Lakhimi cows, which can inform breeding strategies and contribute to the conservation and promotion of this indigenous breed. Understanding the unique reproductive dynamics of Lakhimi cattle shall help the veterinarians to assess the proper timing of artificial insemination and ovulation, and to increase the chances of fertilization and pregnancy by synchronization. A better understanding about the follicular waves enables the breeder to better plan for specific calving seasons, for improved herd management, synchronized calving, and more consistent milk production.

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