

Ultrasonographic Assessment of Ovarian Follicular Cyclicity During the Estrous Cycle in Bangladeshi Zebu Cows (*Bos indicus*)

Mohammad Abdullah-Al Mamun^{1,2}, Md. Mahmudul Alam¹, Marzia Rahman³,
Mir Md. Iqbal Hasan⁴, Aminul Haque¹, Zannatul Maoya¹, Nasrin Sultana
Juyena^{1*}

¹Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Bangladesh

²Department of Livestock Services, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh.

³Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Bangladesh

⁴Department of Physiology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh

Corresponding Author: Dr. Nasrin Sultana Juyena, Professor, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Follicular development during the bovine estrous cycle follows a wave-like pattern that varies among breeds and influences reproductive efficiency. Information on ovarian follicular dynamics in indigenous zebu cows (*Bos indicus*) of Bangladesh remains limited, despite their economic importance in tropical livestock production. This study aimed to characterize ovarian follicular dynamics throughout the estrous cycle in Bangladeshi zebu cows. Fourteen clinically healthy cyclic zebu cows were monitored at the Research Animal Farm, Department of Surgery and Obstetrics, Bangladesh Agricultural University from February 2022 to June 2022. Ovarian activity was assessed by repeated alternate-day transrectal ultrasonography throughout the estrous cycle. The position and diameter of ovarian follicles were recorded at each examination and ovulation was identified by the disappearance of a dominant follicle (>12 mm) followed by the appearance of a corresponding corpus luteum. Bangladeshi zebu cows predominantly exhibited a two-wave follicular pattern occurring in 71.43% (10/14) of cows, whereas 28.57% (4/14) exhibited three-wave cycles. The interovulatory interval was 20 days in two-wave cycles and 21 days in three-wave cycles. Ovulatory follicle diameter averaged 15.92 ± 0.25 mm and 14.92 ± 1.12 mm in two- and three-wave cycles, respectively. Ultrasonography is the gold-standard method for evaluating ovarian function in cattle under both research and field conditions. The ability to repeatedly examine the same animals without causing significant stress provides substantial advantages for studying dynamic reproductive processes.

Keywords: *Bos indicus*; estrous cycle; follicular dynamics; transrectal ultrasonography; zebu cow

I. INTRODUCTION

Efficient reproductive performance is fundamental to the sustainability and profitability of cattle production systems. In tropical countries such as Bangladesh, indigenous zebu cattle (*Bos indicus*) constitute the majority of the bovine population owing to their adaptability to harsh environmental conditions, resistance to endemic diseases, and ability to survive under low-input management systems. Despite these advantages, reproductive inefficiency remains a major constraint limiting productivity, primarily due to poor estrus detection, prolonged calving intervals, and suboptimal breeding management. A comprehensive understanding of ovarian follicular dynamics during the estrous cycle is therefore essential for improving reproductive efficiency and optimizing the application of reproductive biotechnologies in indigenous cattle [1,2]. The bovine estrous cycle is characterized by the sequential growth, selection, dominance, and regression of ovarian follicles in a wave-like pattern. Depending on the breed and physiological status, cows generally exhibit either two or three follicular waves during a single estrous cycle, with each wave initiated by the synchronous emergence of a cohort of small antral follicles. One follicle is subsequently selected as the dominant follicle, while the remaining subordinate follicles undergo atresia. In non-ovulatory waves, the dominant follicle regresses under the suppressive influence

of progesterone secreted by the corpus luteum, whereas the dominant follicle of the final wave ovulates following luteolysis and the preovulatory surge of luteinizing hormone [3,4]. The pattern of follicular wave development directly influences the timing of ovulation and consequently affects fertility, artificial insemination programs, and embryo production efficiency. Advances in transrectal ultrasonography have substantially improved the ability to investigate ovarian physiology in cattle under both experimental and field conditions. Real-time ultrasonography allows repeated, non-invasive monitoring of follicular emergence, growth, deviation, ovulation, and corpus luteum development throughout the estrous cycle. This technique has become the gold standard for studying follicular dynamics and has significantly enhanced the understanding of ovarian function in domestic ruminants [5]. Although follicular wave patterns have been extensively described in *Bos taurus* breeds, comparatively fewer studies have investigated these characteristics in indigenous *Bos indicus* cattle, particularly under tropical production systems where breed-specific physiological differences may exist [6]. Accurate estrus detection remains one of the greatest challenges in cattle reproduction, especially in zebu cows that frequently exhibit shorter estrus duration and less pronounced behavioral signs than European breeds. Failure to detect estrus at the appropriate time reduces conception rates and increases economic losses associated with repeated inseminations and extended calving intervals. Consequently, objective methods capable of supporting conventional estrus detection have attracted considerable research interest [7]. Despite the economic importance of indigenous zebu cattle in Bangladesh, information regarding ovarian follicular wave characteristics and their relationship with vaginal electrical resistance remains scarce. Most available knowledge has been extrapolated from studies conducted in *Bos taurus* cattle or zebu populations raised under different environmental and management conditions. Breed-specific baseline information is therefore necessary for improving reproductive management strategies and supporting the development of efficient estrus detection protocols for indigenous cattle. Therefore, the present study was designed to characterize ovarian follicular dynamics in Bangladeshi zebu cows using repeated transrectal ultrasonography during different stages of the estrous cycle.

II. METHODS

The study was conducted at the Research Animal Farm, Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, between February 2022 to June 2022. The experimental protocol was approved by the Animal Welfare and Experimentation Ethics Committee (AWEEC), Bangladesh Agricultural University (Approval No. AWEEC/BAU/2022 (69)). All experimental procedures complied with institutional guidelines for the care and use of animals in research. A total of 14 clinically healthy, cyclic indigenous zebu cows (*Bos indicus*) were included in the study. All animals exhibited regular estrous cycles, were maintained under uniform management conditions and received routine feeding and husbandry practices implemented at the research farm throughout the experimental period.

Ultrasonographic evaluation of ovarian follicular dynamics

Ovarian follicular dynamics were monitored throughout the estrous cycle by repeated transrectal ultrasonographic examinations performed on alternate days using a ultrasound scanner (MyLab™Five VET, Esaote, Italy) equipped with a linear-array transrectal transducer. During each examination, both ovaries were systematically scanned to identify and record the position, number and diameter of all visible follicles. Individual follicles were traced sequentially across examinations according to their relative ovarian location and size to characterize follicular wave emergence, growth, dominance and ovulation. Ovulation was confirmed by the disappearance of a dominant follicle measuring >12 mm in diameter, followed by the subsequent appearance of a corresponding corpus luteum (CL). The data were arranged in a Microsoft Excel data sheet and IBM SPSS® version 20 was used to statistically evaluate the data collected during routine examinations of the ovaries.

III. RESULTS

Characteristics of ovarian follicular waves

Repeated transrectal ultrasonographic examination successfully monitored ovarian follicular dynamics throughout the estrous cycle in all experimental cows. Two distinct follicular wave patterns were identified: two-wave and three-wave cycles. Among the 14 indigenous zebu cows examined, 10 cows (71.43%) exhibited a two-wave follicular pattern, whereas four cows (28.57%) demonstrated a three-wave pattern. The interovulatory interval (IOI) averaged 20 days in cows with two-wave cycles and 21 days in cows with three-wave cycles. The mean diameter of the ovulatory follicle was slightly greater in two-wave cycles (15.92 ± 0.25 mm) than in three-wave cycles (14.92 ± 1.12 mm). The temporal pattern of dominant follicle development differed between the two follicular wave patterns. In two-wave cycles, the first dominant follicle reached its maximum diameter on Day 11.50 ± 0.50 , whereas the second dominant follicle attained its maximum diameter on Day 13.30 ± 0.42 . In contrast, cows exhibiting three-wave cycles reached maximum dominant follicle diameter earlier, with the first,

second, and third dominant follicles attaining peak size on Days 8.50 ± 0.65 , 8.25 ± 0.25 , and 9.50 ± 1.04 , respectively. Similarly, the maximum diameter of dominant follicles varied slightly between the two follicular patterns. In two-wave cycles, the first and second dominant follicles reached maximum diameters of 15.65 ± 0.18 mm and 15.94 ± 0.24 mm, respectively. In three-wave cycles, the corresponding maximum diameters were 14.28 ± 0.97 mm, 13.93 ± 0.93 mm, and 14.92 ± 1.12 mm for the first, second, and third dominant follicles, respectively. Growth rates of dominant follicles also differed between follicular patterns. The first and second dominant follicles in two-wave cycles exhibited growth rates of 1.20 ± 0.06 mm/day and 1.05 ± 0.05 mm/day, respectively. In comparison, dominant follicles in three-wave cycles showed relatively higher growth rates, averaging 1.48 ± 0.14 , 1.47 ± 0.15 , and 1.39 ± 0.09 mm/day for the first, second, and third follicular waves, respectively. Representative ultrasonographic observations illustrating follicular development in two-wave and three-wave cycles are presented.

Table 1: Characteristics of follicular waves and dominant follicles in two-wave and three-wave cycles in experimental cows

Parameters		Two-wave cycle	Three-wave cycle
		(Mean $\pm$ SEM) (n = 10)	(Mean $\pm$ SEM) (n = 4)
Occurrence of wave		71.43% (10/14)	28.57% (4/14)
Interovulatory interval (IOI)		20	21
Diameter of the ovulatory follicle (mm)		15.92 ± 0.25	14.92 ± 1.12
Day at maximum diameter of the dominant follicle			
	1st dominant follicle	11.50 ± 0.50	8.50 ± 0.65
	2nd dominant follicle	13.30 ± 0.42	8.25 ± 0.25
	3rd dominant follicle		9.50 ± 1.04
Maximum diameter of the dominant follicle (mm)			
	1st dominant follicle	15.65 ± 0.18	14.28 ± 0.97
	2nd dominant follicle	15.94 ± 0.24	13.93 ± 0.93
	3rd dominant follicle		14.92 ± 1.12
Growth rate of the dominant follicle			
	1st dominant follicle	1.20 ± 0.06	1.48 ± 0.14
	2nd dominant follicle	1.05 ± 0.05	1.47 ± 0.15
	3rd dominant follicle		1.39 ± 0.09

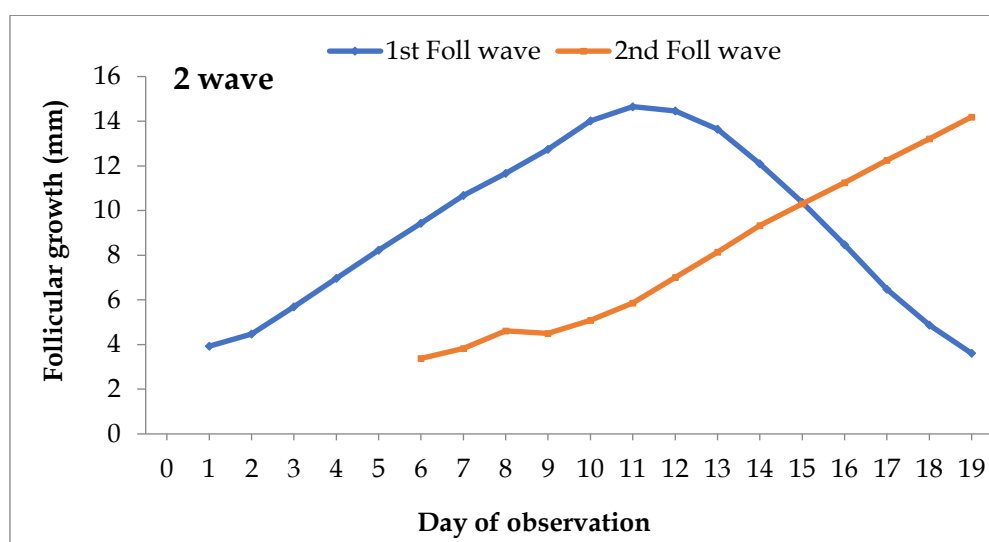


Figure 1: Follicular growth pattern throughout the Two-waved estrous cycle

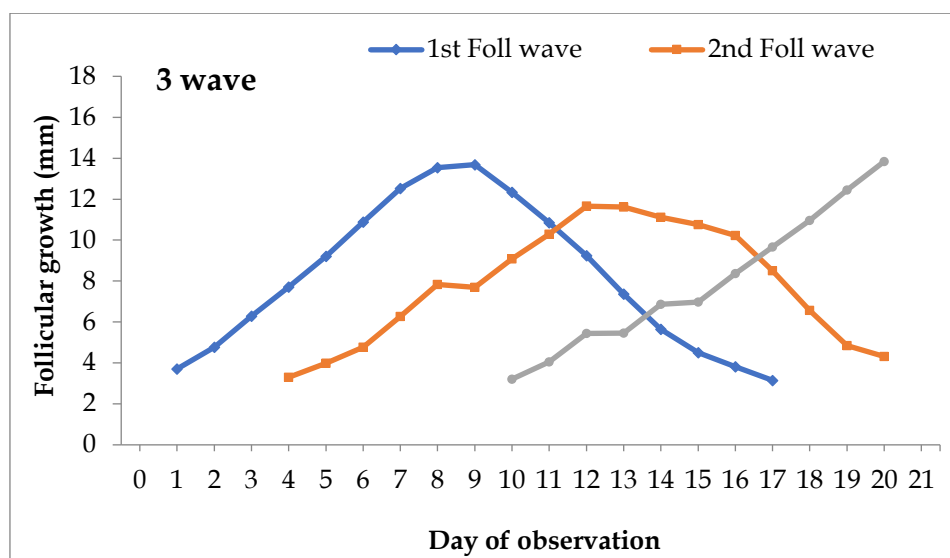


Figure 2: Follicular growth pattern throughout the Three-waved estrous cycle

IV. DISCUSSION

The present study characterized ovarian follicular dynamics throughout the estrous cycle of indigenous Bangladeshi zebu cows (*Bos indicus*). The results demonstrated that two-wave follicular cycles predominated over three-wave cycles, and cyclic fluctuations in VER closely paralleled ovarian activity. These findings provide baseline physiological information for indigenous zebu cattle and contribute to the understanding of reproductive events in tropical production systems. The predominance of the two-wave follicular pattern (71.43%) observed in the present study is consistent with previous reports describing follicular wave dynamics in cattle. Although both two- and three-wave estrous cycles are considered physiologically normal, the frequency of each pattern varies among breeds, age groups, nutritional status, and environmental conditions [8,9]. Earlier investigations reported that two-wave cycles are frequently observed in *Bos indicus* cattle, whereas *Bos taurus* breeds may exhibit either two or three follicular waves with comparable frequency [10]. The predominance of two-wave cycles in the present study therefore appears to reflect the normal ovarian physiology of indigenous Bangladeshi zebu cows. The mean interovulatory interval of 20 days in two-wave cycles and 21 days in three-wave cycles agrees well with the normal duration of the bovine estrous cycle described in previous studies [9,11]. A slightly longer interovulatory interval in three-wave cycles has been consistently reported and is generally attributed to the additional follicular wave that develops before ovulation. The present findings therefore support the concept that the number of follicular waves contributes to variation in estrous cycle length without altering the overall sequence of ovarian events. Ultrasonographic monitoring revealed that ovulatory follicles attained slightly larger diameters in two-wave cycles than in three-wave cycles. Similar observations have been reported in both dairy and beef cattle, where dominant follicles of two-wave cycles often continue growing for a longer period before ovulation than those developing in three-wave cycles. Furthermore, dominant follicles in three-wave cycles exhibited relatively greater daily growth rates than those in two-wave cycles, suggesting more rapid follicular turnover despite achieving slightly smaller maximum diameters. Repeated transrectal ultrasonography proved to be an effective and reliable technique for monitoring ovarian structures throughout the estrous cycle [8,12]. The sequential identification of follicular emergence, growth, ovulation, and corpus luteum formation observed in this study supports previous reports that ultrasonography is the gold-standard method for evaluating ovarian function in cattle under both research and field conditions. The ability to repeatedly examine the same animals without causing significant stress provides substantial advantages for studying dynamic reproductive processes. The present study has certain limitations. The relatively small sample size reflects the intensive nature of repeated ultrasonographic examinations but may limit extrapolation of the findings to the wider zebu population. The findings demonstrate that Bangladeshi zebu cows predominantly exhibit a two-wave follicular pattern and these observations may facilitate improved estrus detection, reproductive monitoring, and the application of assisted reproductive technologies in indigenous cattle under tropical production conditions [13].

V. CONCLUSIONS

This study demonstrated that indigenous Bangladeshi zebu cows predominantly exhibited a two-wave follicular pattern with a normal interovulatory interval of approximately 20–21 days. Transrectal ultrasonography effectively characterized follicular development and corpus luteum formation throughout the estrous cycle.

However, Future research studies should include larger and more diverse populations across different geographical regions of Bangladesh instead of a single research farm. Furthermore, evaluating the applicability of Vaginal Electric Resistance under field conditions and integrating it with ultrasonographic assessments may facilitate the development of more accurate and cost-effective reproductive management strategies for indigenous cattle.

Conflict of Interest: The authors declare no conflict of interest regarding this research.

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