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Follicular dynamics in buffaloes

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Abstract
Buffaloes show seasonality in their reproductive behaviour with diminished performance in the hotter months of the year. A group of eight cyclic healthy buffaloes was selected from breeding herd of buffaloes present at IVRI, Izatnagar. They were examined via trans-rectal ultrasonography to study the pattern of their ovarian activity during the months from April to June, 2021. The average length of the estrous cycle was 20.13±1.67 days with majority of buffaloes showing two-wave pattern followed by one-wave and three-wave pattern. The anovulatory dominant follicle attained maximum diameter 10.78±0.37 mm in duration of 7.60±0.45 days followed by its regression and the onset of a new wave. The ovulatory follicle grew at a faster pace and ovulated after achieving a maximum diameter of 13.18±0.35 mm and later transformed in a corpus luteum achieving a mean maximum diameter of 18.20±0.70 mm by day 12.20±1.26 post-ovulation. Later, the regression of this CL led to the onset of a new wave.

Keywords: follicular dynamics, buffalo, summer season

Introduction

A significant provider of premium animal products like milk, meat, and hide, the water buffalo (*Bubalus bubalis*) is a farm animal species with significant economic value that is raised in tropical and subtropical countries. Zicarelli (1994) [9] states that even in systems with limited input, it can sustain optimal productivity levels. Nearly 49% of all milk produced for the Indian dairy sector comes from buffalo, which number roughly 109.85 million in the country according to the 20th livestock census (Annual report-DAHD, 2018-19) [1]. According to Baruselli *et al.* (1997) [4], they are animals that exhibit polyestrus and seasonality in reproductive patterns. There is a notable decrease in fertility throughout the summer months, but they exhibit increased reproductive activity in the cooler months of the year, with their breeding season lasting from October to March (Baithalu *et al.*, 1997) [2]. There has been documented heterogeneity in the pattern of follicular development and the number of follicular waves in an estrous cycle among buffaloes in various parts of the world.

The most common estrous cycle patterns expressed by buffaloes are two-wave (63.33%), followed by three-wave (33.33%), and one-wave (3.33%). The anovulatory dominant follicles in the two-wave and three-wave patterns had mean maximum diameters of 1.51±0.24 cm and 1.33±0.18 cm, respectively, whereas the ovulatory follicles reached 1.55±0.16 cm and 1.34±0.13 cm, in that order (Baruselli *et al.*, 1997) [4]. On the other hand, three wave pattern (53.6%) of the estrous cycle was more common than two wave pattern (46.4%) in another study on Egyptian buffaloes. The two and three wave patterns of the estrus cycle had mean durations of 22.1±0.6 and 23.7±1.3 days, respectively. The average lifespan of the observed CL was 17.1±0.8 days, consisting of a growth period of 11.2±0.5 days and a regression period of 4.4±0.3 days. Based on 7.0±0.4 days of cycle, the mean maximum diameter of CL was 1.5±0.04 cm (Barkawi *et al.*, 2008) [3]. According to another study on Murrah buffaloes, on 15.22±5.26 and 17.62±1.68 days of the estrous cycle, respectively, for multiparous and heifer buffaloes, CL reached a mean maximum diameter of 19.58±4.16 mm and 17.74±3.32 mm, respectively (Manik *et al.*, 2002) [6]. The purpose of our research was to investigate the summertime follicular dynamics in buffaloes.

Materials and Methods

The experiment was carried out from April to June of 2021 at the ICAR-Indian Veterinary Research Institute, Izatnagar, in the Artificial Insemination Laboratory, Cattle and Buffalo Farm, Livestock Production and Management Section. To evaluate the ovarian activity of the buffalo herd, a veterinary ultrasound scanner (ExaGo-IMV, USA) was used for screening. For the purpose of the study, a group of eight healthy cyclic parous buffaloes were chosen based on the existence of developing follicles on either ovary and the corpus luteum (CL). Throughout the course of an entire estrous cycle, the buffaloes were sonography per-rectally on alternate days.

Results and Discussion

The day of estrus was thought to be the initial point of observation. Large follicle fate was dictated by the state of corpus luteum at that particular moment, and as a result, the follicular waves were classified as either ovulatory or anovulatory waves. The follicular growth pattern had a distinctive wave pattern, which was consistent with earlier research (Baruselli *et al*., 1997; Manik *et al*., 1998) [4, 7]. This first period of follicular recruitment, selection, and dominance was followed by either ovulation or atresia. With an average follicular number of 9.00±0.98, the anovulatory wave began to form 1.33±0.15 days after estrus. For a duration of 18.00±0.71 days, the wave persisted. The largest follicle grew at a rate of 0.68±0.05 mm/day and reached its maximum size in 7.60±0.45 days, measuring 10.78±0.37 mm in diameter. After waiting for a duration of 3.40±0.59 days, the big follicle experienced atresia due to the mature CL's higher levels of circulatory progesterone (>1 ng/ml) that inhibited the generation of LH surge. After continuing to recede at a pace of 0.61±0.09 mm/day, the follicle vanished in 5.80±0.89 days. Monitoring the growth pattern of subordinate follicles was challenging overall, and no subordinate follicle growth more than 5 mm was noted.

With a follicular number of 12.83±1.59, the ovulatory wave was observed to begin after a mean length of 9.20±1.69 days post-estrus. The anovulatory waves of the acyclic and cyclic groups were substantially longer than the wave duration (11.13±0.59 days) (p<0.0001). One follicle performed better than the others in the recruited cohort and reached a preovulatory size of 13.18±0.35 mm in diameter, which was substantially larger than the anovulatory follicles in the acyclic and cyclic groups (p<0.001). Large follicles grew to their maximal diameter at a rate of 0.90±0.10 mm/day and often ovulated, requiring a mean duration of 6.63±0.80 days. Manik *et al*. (1998) [7] found that the dominating follicle in the ovulatory wave had a bigger diameter (13.80±0.37 mm) compared to the anovulatory wave (12.40±0.81 mm).

However, our research showed that ovulatory wave follicles grew at a quicker pace (0.90±0.10 vs. 0.66±0.12 mm/day). In Colombian multiparous Murrah buffalo, Ojeda and colleagues measured the mean maximum diameter of the dominant follicle, which came out to be 17.00±4.60 mm (Ojeda and al., 2014) [8]. At an average development rate of 0.55±0.05 mm/day, the second largest follicle reached a maximum diameter of 6.11±0.09 mm in 3.20±0.29 days. The subordinate follicle's mean maximum size and growth rate were found to be significantly smaller (p<0.0001 and p<0.01, respectively) than those of the acyclic group. Regression at an average rate of 0.54±0.09 mm/day caused the follicle to vanish in 2.40±0.24 days. In cyclic cross-bred cattle heifers, the second largest follicle in the ovulatory wave had a mean maximum diameter of 7.17±0.36 mm as opposed to 7.24±0.19 mm with a two-wave pattern of 0.45±0.03 mm/day on average compared to 0.76±0.09 mm/day, and a three-wave pattern of 0.93±0.07 vs. 0.94±0.05 days on average throughout the regression period (Kumar, 2016) [5].

The average length of the estrous cycle was discovered to be 20.13±1.67 days, with a wide range from nine to twenty-five days. On the day of estrus, the mean number of follicles was 5.12±1.07. In this study, the majority of the buffaloes (n = 6, 75.00%) showed a two-wave pattern of the estrous cycle, with one buffalo showing a one-wave (12.50%) pattern and one buffalo displaying a three-wave (12.50%) pattern. This finding is consistent with nearly every study conducted on riverine buffaloes (Baruselli *et al*., 1997, Manik *et al*., 1998, Ojeda *et al*., 2014) [4, 7, 8], with the exception of studies on Mediterranean buffaloes where three wave pattern predominance has been reported (Barkawi *et al*., 2008) [3]. Summary of all the observations recorded in buffaloes exhibiting different patterns of estrous cycles is shown in Table 1.

With a mean luteal diameter of 11.40±0.73 mm, the corpus luteum was first seen on day 1.25±0.16. After reaching a mean maximum diameter of 18.20±0.70 mm on day 12.20±1.26, the diameter was constant for an additional 4.75±1.16 days. The CL displayed a daily growth rate of 0.75±0.19 mm. Starting on day 14.50±1.67, luteal regression progressed at a rate of 1.02±0.16 mm/day. On the day of estrus, the average luteal diameter measured 12.00±0.58 mm. Previous research on riverine buffaloes (Barkawi *et al*., 2008; Ojeda *et al*., 2014) [3, 8] also found similar observations. The corpus luteum was shown to endure for an average of 20.00±1.66 days.

To sum up, the dynamics of ovarian follicles in buffalo and cattle are comparable. Buffalo had both 3-wave and 1-wave cycles, despite the 2-wave cycle being the most prevalent. The length of the estrous cycle and the luteal phase are related to the number of waves in a cycle.

Table 1: Follicular dynamics in Adult cyclic Murrah Buffaloes during non-breeding season (n=8)

S. No.	Parameters	1-wave	2-wave	3-wave
1	Proportion of animals (%)	12.500 (1/8)	75.000 (6/8)	12.500 (1/8)
2	Duration of estrous cycle (days)	9.000	19.860±1.908	22.000
3	**Anovulatory wave 1**			
	a) Wave length (days)		18.800±0.663	15.000
	b) Wave onset (day)		1.400±0.245	1.000
	c) No. of follicles at wave onset		9.467±1.467	6.000
	d) Growth rate (mm/day)		0.670±0.088	0.506
	e) Maximum diameter (mm)		11.190±0.349	8.250
	f) Days elapsed to attain maximum size (days)		7.920±0.638	8.000
	g) Static phase (days)		3.080±0.898	2.000
	h) Regression rate (mm/day)		0.556±0.135	0.600
4	**Anovulatory wave 2**			
	a) Wave length (days)			15.000
	b) Wave onset (day)			9.000
	c) No. of follicles at wave onset			8.000

	d) Growth rate (mm/day)			0.750
	e) Maximum diameter (mm)			8.700
	f) Days elapsed to attain maximum size (days)			6.000
	g) Static phase (days)			5.000
	h) Regression rate (mm/day)			0.843
5	**Ovulatory wave**			
	a) Wave length (days)	11.000	11.520±0.498	8.000
	b) Wave onset (day)	-1.000	8.133±1.984	15.000
	c) No. of follicles at wave onset	12.000	12.470±2.080	16.000
	d) Growth rate (mm/day)	1.050	0.833±0.085	1.450
	e) Growth period (days)	10.000	7.078±0.745	8.000
	f) Pre-ovulatory diameter (mm)	13.000	13.200±0.397	13.000
6	No. of follicles at estrus	6.000	4.688±1.048	12.000

Day of estrus was considered as Day 0.

Conclusion

The study conducted at the ICAR-Indian Veterinary Research Institute, Izatnagar, focused on evaluating the ovarian activity of buffaloes using a veterinary ultrasound scanner. Through sonographic observations, the follicular growth pattern was monitored during the estrous cycle. Results revealed distinct follicular wave patterns, with the ovulatory wave displaying quicker growth rates compared to anovulatory waves. The study also highlighted variations in follicular sizes, growth rates, and regression periods, emphasizing the complexity of follicular dynamics in buffaloes. These findings contribute to a better understanding of buffalo reproductive physiology, showcasing similarities and differences with cattle. Overall, the research provides valuable insights into the ovarian dynamics of buffaloes, shedding light on their reproductive patterns and cycles.

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