

associated with poor reproductive performance in buffaloes.

Keywords: Murrah buffalo, breeding season, corpus luteum, progesterone

Buffaloes hold significant importance in the dairy industry, particularly in the tropical and subtropical regions, and are crucial for supporting the rural economy in developing countries globally (Perera, 2011). In recent times, there has been a steady increase in the buffalo population owing to the growing worldwide demand for both meat and milk (Warriach *et al.*, 2015). In the tropics, buffalo breeding season is typically observed from September to February (Prakash, 2002), with a limited number of buffaloes exhibiting cyclicity during April (Taneja, 1998). In tropical regions with the influence of increasing photoperiods, the occurrence of rainfall, quality and availability of fodders, and management practices play a significant role in shaping the reproductive patterns in buffaloes (Roy and Prakash, 2007). The estrous cycle is regulated by the hypothalamus-pituitary-ovarian axis, marked by two or three distinct follicular waves and dynamic changes in the CL (Bhattacharyya *et al.*, 2016).

The main function of the CL is to produce P_4 , which is essential for preparing the uterus for embryonic development, implantation, and sustaining pregnancy (Arosh *et al.*, 2004). The CL function is tightly regulated by luteotropic factors, such as luteinizing hormone, angiogenic growth factors, adipokines, and neuropeptides, and the production of luteolytic factors, prostaglandin $F_{2\alpha}$ in the endometrium (Mlyczynska *et*

al., 2022). Any disruptions in its physiological processes can lead to recurrent pregnancy loss and infertility leading to a significant economic loss. In buffaloes, embryonic mortality rates are significantly higher during periods of extended daylight hours, ranging from 20 per cent to 40 per cent, as indicated by Campanile *et al.* (2005), compared to shorter daylight periods, where the mortality rate is only 7 per cent (Baruselli, 1997). Buffaloes going through the transition period towards non-breeding season seemed to experience a gradual decrease in CL activity over consecutive cycles before entering into summer anestrus (Campanile *et al.*, 2010). However, there is no comprehensive study that reported the impacts of seasonality on CL dynamics and P_4 profiling in buffaloes in an organized farm set-up. In this study, we investigated the CL dynamics and serum P_4 concentrations in the parous Murrah buffaloes during both breeding and non-breeding seasons.

Materials and Methods

The study was carried out at the cattle and buffalo farm, ICAR-Indian Veterinary Research Institute, from May to December 2023. A total of twelve parous Murrah buffaloes aged 4 to 8 years were included in the study. These buffaloes were subjected to monitoring of luteal dynamics for one estrous cycle during the breeding (n=6) and non-breeding season (n=6). Animals were managed in a loose house system following the standard guidelines of the Institute Animal Ethics Committee. Trans-rectal ultrasonography (USG) was performed daily throughout one estrous cycle for each buffalo using a B-mode linear array transducer, frequency of 7.5 MHz

Table I. Effect of season on CL dynamics and P_4 concentration in buffaloes

Attributes	Winter (n=6)	Summer (n=6)	P value
Average CL diameter (mm)	16.19±0.24	15.61±0.42	0.23
Maximum CL diameter (mm)	19.64±0.28	18.80±0.44	0.14
Attainment of maximum CL diameter (day)	13.17±0.40	12.83±0.30	0.52
Static CL diameter (mm)	18.79±0.42	18.56±0.40	0.71
Growth rate (mm/day)	0.70±0.04	0.58±0.05	0.10
Regression rate (mm/day)	0.89±0.06	0.69±0.06	0.06
Onset of CL regression (day)	16±0.25	14.83±0.40	0.03
Average P_4 concentration (ng/mL)	3.85±0.26	3.46±0.24	0.30
Peak P_4 concentration (ng/mL)	7.57±0.34	6.41±0.39	0.04

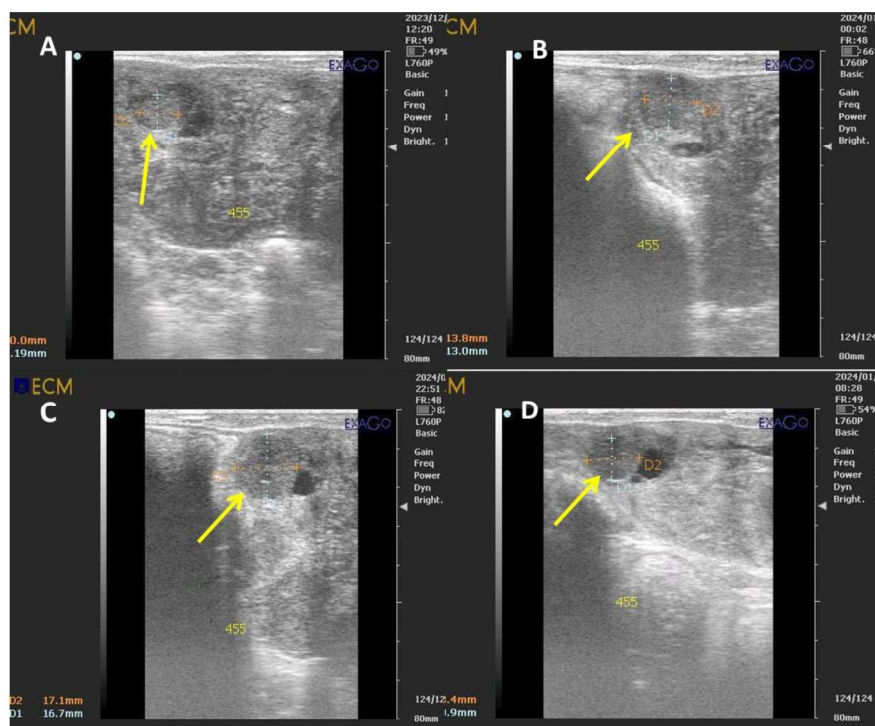


Fig.1. Sonographic imaging of buffalo corpus luteum (CL). A) Developing CL on day 3. B) CL on day 12 C) Developed CL on day 15 D) Regressing CL on day 18

(ExaGo, USA). The longitudinal and transverse diameter of the CL was measured (Fig.1). Blood samples (3 ml) were collected in clot activator tubes (BTL research labs, Gujarat, India) for separation of serum after centrifugation at 3000 rpm for 15 minutes. The serum samples were stored at -20°C .

Serum P_4 estimation

Serum P_4 concentration was estimated using solid phase enzyme-linked immunosorbent assay (Cat# FR E-2500R, LDN, GmbH, Germany). The detection range was 0.14 to 40 ng/mL, and the sensitivity was 0.045 ng/mL. The intra-assay and inter-assay coefficient of variations were $<7\%$ and $<10\%$, respectively.

Statistical analysis

All data were analyzed by Graph Pad Prism 8.0.2. The CL diameters, growth and regression rate, and serum P_4 concentration were compared between breeding and non-breeding seasons using an independent 't' test. The association of CL diameter and P concentration was analyzed using Pearson correlation. The level of signifi-

cance was set at $P < 0.05$.

Results and Discussion

Effect of season on luteal dynamics

The average CL diameter and maximum CL diameter were observed to be relatively greater during the breeding season compared to the non-breeding season (Fig.2. A and B; Table I). Day of maximum CL diameter and static CL diameter also showed similar trends across the groups. However, the CL growth and regression rate was relatively higher during the breeding season than non-breeding season. This is also indicative of more efficient reproductive function during winter or breeding season than summer or non-breeding season. A study conducted by Ali (2015) revealed a reduction in the size of large follicles and mature CL during the summer, in contrast to the winter. Lower-quality nutrition and heat stress experienced during the summer may have contributed to these changes (Ali, 2015). Our study also revealed that average day of CL regression started earlier during the non-breeding season (14.83 ± 0.40 vs 16 ± 0.25 , $P < 0.03$) than the breeding season (Fig.3. A;

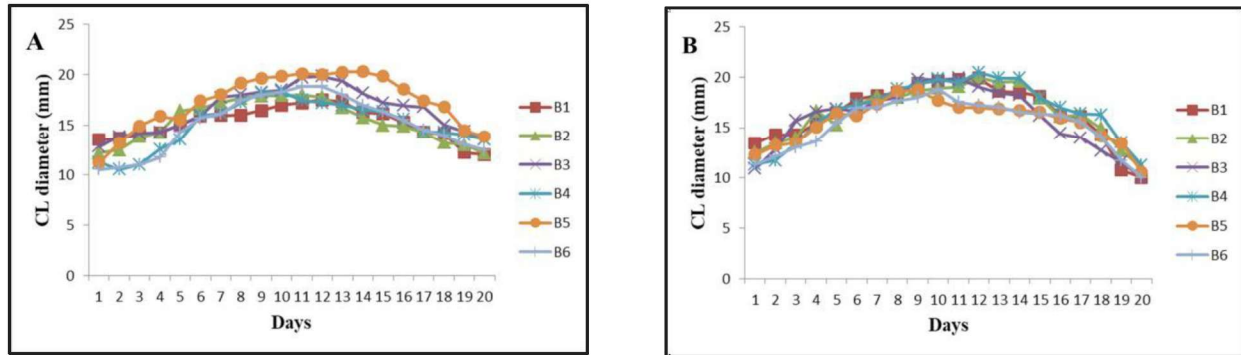


Fig.2. Line diagram showing the growth pattern of CL throughout the estrous cycle during summer (A) and winter (B) season

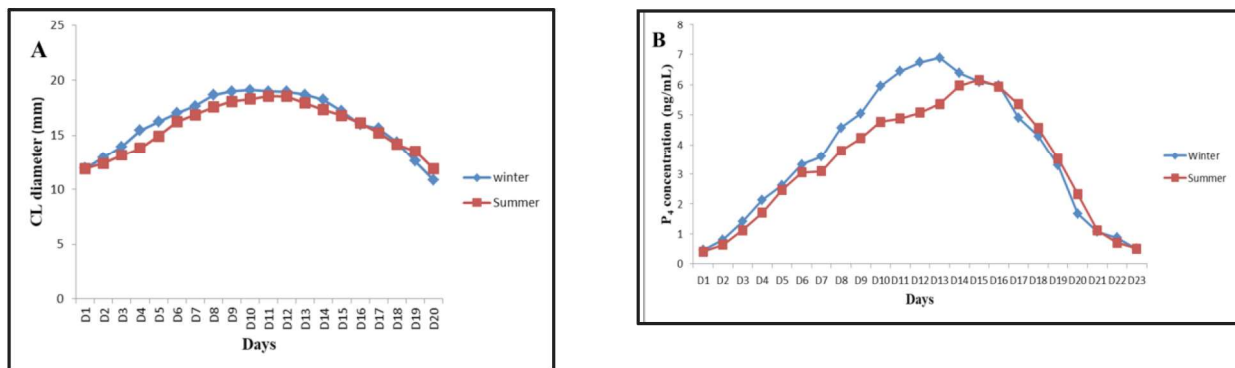


Fig.3. A. Line diagram showing the average CL diameter throughout the estrous cycle during summer and winter season (B) Peak average P₄ concentration throughout the estrous cycle during summer and winter season.

Table I). This could be due to the exposure to high temperatures during summer, which adversely affects the biological functions of buffaloes, leading to decreased enzyme reactions, hormonal secretions, and blood metabolites (Marai and Habeeb, 2010). An increased secretion of PGE₂ and PGF_{2α} from bovine endometrial stromal cells in response to heat stress during the summer months has been reported and this may disrupt the normal estrous cycle, leading to infertility in cows (Sakai *et al.*, 2018). However, the exact mechanism of early luteolysis during summer has to be explored in detail in buffaloes.

Effect of season on P₄ secretion

During the non-breeding season out of six buffaloes, one had a supra-basal concentration of P₄ and the other two had a slow rise of P₄, its concentration remained below 2 ng/mL till day 5 of the estrous cycle. The peak P₄ concentration during the breeding season was significantly greater than the non-breeding season (7.57 ± 0.34 vs. 6.41 ± 0.39 ng/mL, P < 0.04, Fig. 3.B) indicating

the sub-optimal luteal development and function during the non-breeding season. In a study conducted by Qureshi *et al.* (2000), they reported that milk P₄ levels during the non-breeding season were found to be lower compared to the normal breeding season under field conditions in postpartum buffaloes. The correlation between CL size and serum P₄ concentration showed a strong positive correlation (r = 0.94, P < 0.001) in the breeding season and non-breeding season (r = 0.73, P < 0.001). This positive correlation between the CL size and P₄ production indicates that the persistence or regression of the CL directly affects the pregnancy of the animal, as the CL continuously produces P₄, which is essential for maintaining pregnancy (Chaudhari *et al.*, 2022). Buffaloes bred during the breeding season show higher pregnancy rates of 58.0 per cent and lower embryonic mortality rates of 7.3 per cent, whereas those bred during the transition to the non-breeding season exhibit pregnancy rate and embryonic mortality rates of 45.6% and 23.0%, respectively (Di Francesco *et*

al., 2012). Thus, the reduced functionality of the CL during the non-breeding season potentially hindered the pregnancy rates by reducing the capacity for embryonic attachment and increasing embryonic mortalities (Russo *et al.*, 2010).

Conclusions

From this study, it can be concluded that although the CL development during breeding and non-breeding season in Murrah buffaloes was at par, its regression occurs faster during the non-breeding season. However, slow rise and lower peak serum P4 concentration were indicative of luteal insufficiency during the non-breeding season in Murrah buffaloes. Understanding the factors influencing CL development and luteal steroidogenesis during non-breeding season is essential for developing any strategy to address luteal insufficiency in buffaloes. Further research on the adverse effects of environmental stressors on CL functions is warranted to improve the reproductive performance in buffaloes during the unfavourable breeding season.

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