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Insights of structural and functional luteolytic pattern in Tharparkar cows (*Bos indicus*) through doppler ultrasonography

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ABSTRACT

The study aimed to investigate real-time changes in intraluteal blood flow, progesterone (P4) profile, and associated structural alterations in the corpus luteum (CL) of Tharparkar cows following prostaglandin F2α (PGF2α) administration. Tharparkar cows (n = 8), at least 60 days postpartum with a well-developed CL, were administered cloprostenol (500 µg, intramuscularly). Ultrasonographic examinations and blood sampling were performed at 0, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h post-injection. CL diameter remained unchanged up to 4 h post-treatment, followed by a significant reduction ($p < 0.05$) beginning at 12 h and progressing through 24, 48, and 72 h, indicating structural luteolysis. The mean CL diameter decreased from 20.29 ± 1.19 mm at 0 h to 11.55 ± 0.30 mm at 72 h. An acute increase in intraluteal blood flow was observed, peaking at 1 h post-injection, followed by a significant decline ($p < 0.05$), with the lowest values recorded at 72 h. Serum P4 concentrations decreased significantly ($p < 0.05$) from 1 h onward, confirming functional luteolysis. The findings demonstrated a biphasic vascular response characterized by an initial rise followed by a sustained decline in blood flow, coupled with progressive structural regression of the CL. The study provided important insights into the hemodynamic and functional dynamics of PGF2α-induced luteolysis in *Bos indicus* cows.

Keywords*:* Blood flow, Color Doppler USG, Luteolysis, Prostaglandin (PGF2α), Tharparkar

Cattle, especially native breeds are essential for reducing rural poverty and employment generation. *Bos indicus* cattle, particularly those found in Southeast Asia are climatically suited to the hot and humid conditions of the tropics (Sodhi *et al.* 2013). *Bos indicus* cattle are mostly recognized for their ability to withstand heat and disease (Glass *et al.* 2005, Sahu *et al.* 2025) as well as their ability to thrive in low input production systems while still maintaining satisfactory production levels in subtropical and tropical areas. Efforts to maximize the superior indigenous cattle were made possible by incorporating advanced reproductive biotechnologies. PGF2α is a crucial part of synchronization between donors and recipients in embryo transfer programs. It induces luteolysis within hours to a few days following administration, leading to regression of the corpus luteum (CL) and onset of estrus in Ovsynch- and CO-Synch–based protocols (Stevenson and Phatak, 2010). The mechanisms by which PGF2α acts on early-stage (recently ovulated) CL are not fully understood (Cuervo-Arango *et al.* 2011).

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In domestic animals such as cattle, PGF2α, and its analogues act as luteolytic agents. They typically trigger estrus when administered during the luteal phase of estrous cycle (Smith *et al.* 1998). Following ovulation, the newly developed CL originates from the remnants of the ruptured Graafian follicle and exhibits significant vascularization within 2-3 days after ovulation (Reynold *et al.* 2000). The early CL resists luteolytic dose of PGF2α (Tsai and Wiltbank, 1998). In mature CL, vascular endothelial cells constitute up to 50% of the total cell population. During this time, the CL develops and gains the ability to undergo luteolysis in response to PGF2α. Prostaglandin is associated with variation in intraluteal bloodstream (Miyamoto *et al.* 2005). Early signs of luteolysis include a decrease in luteal cross-sectional area, a fall in blood P_4 levels, and luteal cell degranulation (McCracken *et al.* 1979). Also, the early histological changes detected during luteolysis predominantly change the vascular components of the CL (Bollwein *et al.* 2012). During luteolysis, a positive relationship exists between the reducing ovarian bloodstream and the systemic P_4 concentration within dairy animals (Herzog *et al.* 2010, Sahu *et al.* 2026c). The role of Color Doppler ultrasonography in monitoring luteal blood flow is a crucial factor in understanding PGF2α induced luteolysis in cows.

Color Doppler ultrasonography is a useful and non-invasive technique for evaluating ovarian vascular function and makes it possible to see variations in the bloodstream in a particular region of the CL (Miyazaki *et al.* 1998, Acosta *et al.* 2003). This method can assess the CL function and comprehend the hemodynamic factors involved in the luteolytic process (Figueira *et al.* 2015). There is substantial evidence that PGF2α increases local blood flow to mature CL. However, detailed information on the variation in intraluteal bloodstream in *Bos indicus* is still lacking. Thus, the current study focused on investigating the real-time variation in intraluteal blood flow and corresponding structural changes after the PGF2α analogue with the help of Color Doppler ultrasonography.

MATERIALS AND METHODS

Location and management: All Tharparkar cows were maintained at the Cattle and Buffalo Farm, ICAR–IVRI, Izatnagar, India under standard management conditions. The animals were provided with 3.5–4.5 kg concentrate feed containing 20% digestible crude protein and 70% total digestible nutrients, along with routine feeding and management practices.

Ethical approval: All experimental procedures were conducted in accordance with ARRIVE 2.0 guidelines and were approved by the Institutional Animal Ethics Committee of ICAR–IVRI, Izatnagar, Uttar Pradesh, India (Approval No.: 26-3/2020-21/JD(R)/IAEC).

Color Doppler ultrasonography and experimental design: Eight Tharparkar cows (n = 8) of 2–4 parity and ≥60 days postpartum, with a body condition score of 3.5–4.0 (on a 1–5 scale), were selected based on the presence of a well-developed CL as confirmed by transrectal ultrasonography (Exago ECM, France) and retrospective ovulation records. The sample size (n = 8) was based on the availability of animals meeting the selection criteria and is comparable to previous Doppler ultrasonography studies in cattle. The repeated-measures design, where each animal served as its own control, enhanced statistical power by reducing inter-animal variability. Each animal served as its own control, with observations recorded at 0 h (prior to PGF2α administration) considered as baseline values for subsequent comparisons. After confirmation of the CL, cloprostenol (500 µg; Estrumate™, MSD Animal Health, India) was administered intramuscularly. Ultrasonographic examinations were performed using a 7.5 MHz transrectal linear transducer (Exago ECM, France) in B-mode and Color Doppler modes. Color Doppler settings were standardized throughout the study, with color gain maintained at approximately 20 dB and pulse repetition frequency (PRF) set at 4000 Hz to avoid aliasing. The insonation angle was kept ≤60° (mean ~50°) during all examinations (Sahu *et al.* 2026a). When aliasing occurred, PRF and scale were adjusted and the insonation angle was reconfirmed to ensure accurate signal acquisition. For evaluation of blood flow distribution within the CL, cross-sectional images were obtained at three standardized planes, namely apex, middle, and base as described earlier (Sahu *et al.* 2026d). The apex was defined as the superficial portion of the CL closest to the ovarian surface, the base as the deeper portion adjacent to the ovarian stroma, and the middle as the intermediate section between apex and base. These planes were identified by gradual transducer manipulation along the longitudinal axis of the ovary, ensuring consistent orientation and complete coverage of the CL structure. Care was taken to maintain uniform probe positioning and minimal variation in scanning angle across examinations to ensure reproducibility of measurements. Ultrasound scans were performed immediately before treatment (0 h) and subsequently at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h post-administration (Fig. 1) to monitor the luteolytic pattern. Images were recorded only when clear and consistent Doppler signals were obtained, and measurements were based on stable image frames to minimize variability. All scans were performed by a single experienced operator using a standardized protocol to minimize inter-operator variability. Consistent probe positioning and scanning technique were maintained across all animals and time points to reduce potential operator bias. All Doppler examinations were conducted by a trained operator using predefined machine presets. Reliability assessment demonstrated high reproducibility of measurements (intra-observer ICC = 0.87, inter-observer ICC = 0.83). All Doppler images were quality-checked, and recordings affected by artifacts (e.g., motion or aliasing) were excluded and reacquired to ensure data accuracy and consistency.

Blood samples were collected at each designated time

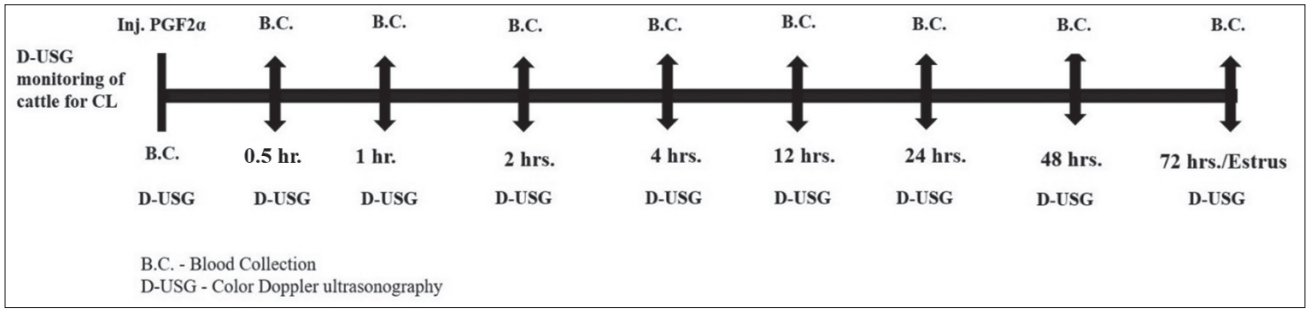

Fig.1. Schematic design of the experimental protocol showing blood collection and Color Doppler ultrasonography at standardized time intervals (0, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h).

point for the determination of serum P_4 concentrations. Upon identification of the corpus luteum (CL), the ultrasonographic image was frozen at its maximum cross-sectional area, and the longest longitudinal (D_1) and transverse (D_2) diameters were measured, from which CL dimensions were calculated using standard geometric relationships: Radius (R) = $(D_1 + D_2)/4$; CL area (mm^2) (A) = $\pi \times (D_1/2) \times (D_2/2)$; and CL volume ($mm^3$) (V) = $(4/3) \times \pi \times R^3$ (Miyazaki *et al.* 1998; Sahu *et al.* 2026a). The vascularization area within the CL was assessed using the Color Doppler mode of an ultrasound equipped with a 7.5-MHz linear transducer. During each ultrasonographic examination, in Color Doppler mode, blood flow toward the transducer (red) and away from the transducer (blue) was delineated using the magnetic lasso tool in Adobe Photoshop CC (Version 26.6, 2025) to determine the area in pixels (Sahu *et al.* 2026a). To ensure standardization, all images were acquired using identical ultrasound settings (gain, PRF, depth, and zoom) and exported at a uniform resolution and scale. The region of interest (ROI) was consistently defined as the entire visible CL boundary, and only clearly demarcated color signals representing blood flow were selected using predefined selection criteria. To minimize observer-related bias, repeated measurements were performed on a subset of images, and consistency in selection was ensured. Images with poor quality or artifacts were excluded and re-acquired. Pixel values were directly comparable across images as all images were captured and processed under identical acquisition settings without variation in resolution or magnification; therefore, no additional normalization was required. The percentage of the colour area in the CL was calculated by dividing the colour area by the total CL area (Sahu *et al.* 2026a).

Progesterone assay: Blood samples were taken by jugular venepuncture using a sterile 18 G' needle collected in a serum clot activator vacutainer. Immediately after blood collection, the samples were kept undisturbed for 30 min. at room temperature and finally centrifuged for 5 min, at 3000 rpm. Then the serum was separated and stored at -20°C until further use. Serum P_4 concentration (ng/ml) was estimated using a P_4 ELISA Kit protocol manufactured by DRG International Inc. GmbH, Marburg, Germany. Intra-assay and Inter-assay coefficient of variations were < 8% and <10% respectively.

Statistical analysis: The diameter, area, volume of the CL, and serum concentration of P_4 are presented as means ±SEM. The portion of the coloured area within the CL that exhibited detectable blood flow was represented as a percentage of the total CL area. The data collected in this study was analysed using the Graph Pad Prism 11.0.0 software module as described earlier (Sahu *et al.* 2026b). The mean values were compared using one-way ANOVA at different time intervals and significance was assessed through post-hoc Tukey testing.

RESULTS AND DISCUSSION

The images of blood flow in cross-sectional planes at the apex, middle, and base, as well as the overall CL, showed similar changes. The proportion of the colored area seen in a vertical picture at the CL's widest point from top to bottom was used as a quantitative metric to depict changes in blood circulation inside the CL.

Variation in the diameter, area, and volume of the CL: Following PGF2α administration in Tharparkar cows, all structural parameters of the CL (diameter, area, and volume) exhibited a similar pattern of significant variation ($p < 0.05$) over time. No significant change was observed up to 4 h post-injection; however, a significant decline began at 12 h and progressed through 24, 48, and 72 h, indicating structural luteolysis. The mean CL diameter decreased from 20.29 ± 1.19 mm at PGF2α injection to 11.55 ± 0.30 mm at 72 h. Correspondingly, CL area and volume also declined significantly, from 324.27 ± 36.35 mm^2 and 4602.77 ± 841.36 mm^3 at 0 h to 104.02 ± 5.54 mm^2 and 809.71 ± 64.17 mm^3 at 72 h, respectively. The CL volume showed a strong positive correlation (r = 0.9126) with the corresponding P4 concentrations.

Variation in intraluteal blood flow area and P4 concentration: Before PGF2α administration, each cow was regarded as its own control at time 0 h, and the same baseline value was consistently used for comparison across all subsequent time points following PGF2α administration. The CL colour area (measured in pixels, where pixel count represented the quantified area of Doppler colour signals corresponding to moving blood within the corpus luteum, as obtained through image-based analysis) also varied significantly ($p < 0.05$) post-PGF2α administration. At 1 h, the CL colour area reached its highest point (2744.17 ± 287.72 px) before dropping to 95.33 ± 3.53 px by 72 h. The colour percentage also showed a similar pattern, peaking at 1 h (19.85 ± 0.53%) and then decreasing significantly to 3.44 ± 0.33% by 72 h (Fig. 2).

Serum P_4 concentrations were highest at PGF2α injection, followed by a significant decrease noted thereafter, dropping from 3.97±0.08 ng/ml at PGF2α injection time to 0.45±0.06 ng/ml at 72 hrs. This progressive decline in CL characteristics and P_4 levels indicated the regressive effect of PGF2α on luteal function, moving towards the day of estrus (Table 1.). The CL volume showed a strong positive correlation (r = 0.9126, Pearson's correlation, $p < 0.05$) with P_4 concentrations. A moderate positive correlation (r = 0.6886, Pearson's correlation, $p < 0.05$) was observed between CL colour area and P_4 concentrations.

In the present study, a biphasic pattern of intraluteal blood flow was evident in Tharparkar cows following PGF2α administration, with an initial increase at 1 h followed by a progressive decline up to 72 h. This pattern was associated with a concurrent decrease in serum P4 concentrations from 1 h onward, suggesting the onset of functional luteolysis prior to structural regression. The early increase in blood flow may reflect a transient vasodilatory response within the corpus luteum, facilitating the action of luteolytic mediators such as prostaglandins, endothelin-1, and nitric oxide. Thereafter, the decline in blood flow

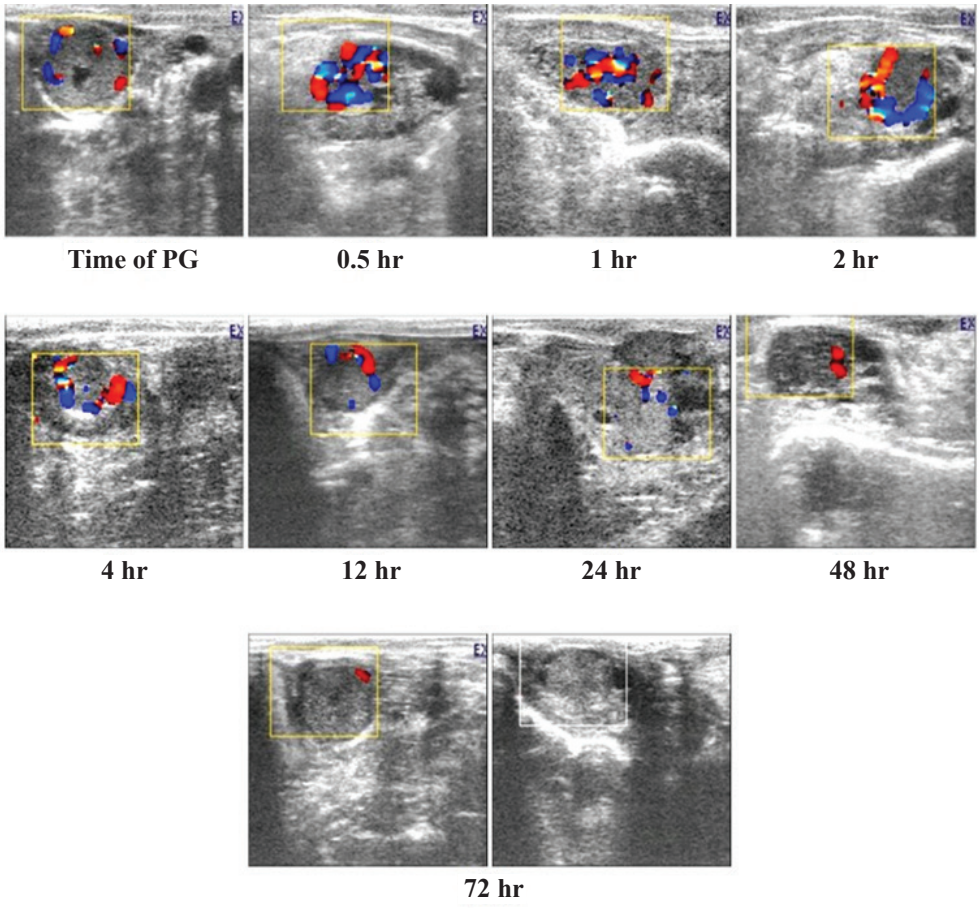

Fig. 2. Doppler luteolytic pattern of Tharparkar cow (*Bos Indicus*) following single dose PGF2α administration

Table 1. Luteolysis pattern and serum progesterone profile of Tharparkar cows following single dose PGF2α administration

Parameter	0 h	0.5 h	1 h	2 h	4 h	12 h	24 h	48 h	72 h
CL diameter (mm)	20.2 ± 0.6^{a}	20.3 ± 0.2^{a}	20.3 ± 0.5^{a}	20.2 ± 0.2^{a}	20.2 ± 0.5^{a}	17.9 ± 0.3^{ab}	15.4 ± 0.1^{bc}	13.5 ± 0.7^{bc}	11.5 ± 0.3^{c}
CL area (mm²)	324.0 ± 36.3^{a}	325.3 ± 36.7^{a}	325.2 ± 35.9^{a}	325.1 ± 36.2^{a}	321.8 ± 35.6^{a}	251.5 ± 9.4^{ab}	187.2 ± 2.2^{bc}	145.4 ± 16.0^{bc}	104.0 ± 5.5^{c}
CL volume (mm³)	4602.7 ± 841.3^{a}	4615.1 ± 816.2^{a}	4606.6 ± 827.7^{a}	4602.3 ± 836.2^{a}	4553.6 ± 844.8^{a}	3030.9 ± 172.0^{ab}	1937.2 ± 33.6^{bc}	1355.3 ± 218.8^{bc}	809.7 ± 64.2^{c}
CL area (px)	9850.5 ± 3.0^{b}	11627.1 ± 616.0^{b}	15251.8 ± 910.2^{a}	11213.5 ± 384.3^{b}	9645.8 ± 188.6^{bc}	8941.6 ± 163.4^{c}	8313.8 ± 219.6^{cd}	6381.5 ± 176.4^{d}	2807.0 ± 205.9^{e}
CL colour area (px)	835.5 ± 29.6^{de}	2256.6 ± 142.7^{ab}	2744.1 ± 287.7^{a}	2019.1 ± 236.5^{b}	1651.6 ± 145.4^{bc}	1172.3 ± 52.3^{cd}	713.5 ± 26.4^{de}	338.5 ± 20.5^{ef}	95.3 ± 3.5^{f}
CL colour (%)	8.5 ± 0.5^{c}	9.5 ± 1.3^{a}	19.8 ± 0.5^{a}	18.0 ± 0.3^{a}	15.6 ± 1.3^{bc}	13.1 ± 0.5^{b}	8.6 ± 0.4^{c}	5.3 ± 0.3^{cd}	3.4 ± 0.3^{d}
P4 concentration (ng/ml)	3.9 ± 0.0^{a}	3.6 ± 0.1^{a}	3.04 ± 0.1^{b}	2.7 ± 0.2^{b}	2.2 ± 0.1^{c}	1.4 ± 0.1^{d}	1.2 ± 0.1^{de}	0.7 ± 0.1^{ef}	0.4 ± 0.1^{f}

Values are expressed as mean ± SEM. Means bearing different superscripts within a row differ significantly ($P < 0.05$).

along with reductions in CL diameter, area, and volume from 12 h onward indicates progression towards structural luteolysis, accompanied by diminished luteal function. The observed positive correlation between CL volume and P4 concentration ($r = 0.9126$, Pearson's correlation, $p < 0.05$) further supported the relationship between luteal structural integrity and progesterone secretion. The temporal pattern observed is comparable to earlier reports in Bos taurus cattle, indicating that the fundamental mechanisms of luteolysis are conserved across breeds. However, the defined sequence and magnitude of vascular and hormonal changes observed in Tharparkar cows provided additional insight into luteolytic dynamics under tropical conditions, with potential implications for improving reproductive

management strategies in indigenous cattle.

In this study, we observed an abrupt rise in blood flow inside the CL after administering a luteolytic dose of PGF2α analogue during the mid-luteal phase of estrous cycle. These findings aligned with previous studies on Holstein cows (Acosta *et al.* 2002) and other *Bos taurus* (Miyamoto *et al.* 2005), and were also observed in Vrindavani crossbred cows (Sahu, 2022). Luteolysis involves a reduction in luteal tissue area due to PGF2α, which reduces large and small luteal cells (Ginther, 2007, Herzog *et al.* 2010). The results indicated that an initial rise in intraluteal blood flow, caused by PGF2α administration in midcycle CL, may initiate the luteolytic process in Tharparkar cows (Sahu *et al.* 2026a).

PGF2α analogue triggered an acute release of PGE2 and PGF2α from the midcycle CL in cows (Hayashi, 2001). The same PGF2α injection acutely induced the expression of cyclooxygenase (COX)-2 from 1 to 4 hrs post-injection in cows (Levy *et al.* 2000). These observations indicated that the acute increase in blood flow inside the midcycle CL may be caused by prostaglandin synthesis from arachidonic acid and the enzymatic activity of COX-2, which includes the vasodilatory prostacyclin (PGI2). Nitric oxide (NO) acts like a local vasodilator and might have a direct role in the luteolytic process when the CL begins to regress (Pate and Keyes, 2001). During luteolysis, the arrival of immune cells, especially macrophages, suggested an inflammatory-like state linked to the regression of the CL. These immune cells release substances that promote luteal cell death (Shirasuna and Miyamoto, 2017). According to Schams and Berisha (2004), the cessation of angiogenic growth factors like vascular endothelium growth factor plays a role during structural luteolysis.

Interestingly, changes in blood vessels during the regression of CL first showed a quick jump in blood flow, which could be seen as a rise in color area of the midcycle CL. These vascular changes mattered a lot for releasing vasoactive peptides like endothelin-1 (ET-1) and angiotensin II (Ang-II). Ultimately, this led to lesser blood flow because of vasoconstriction. The sudden rise in the bloodstream inside the luteal tissue shortly after PGF2α injection, from 0.5 to 2 hrs is vital for triggering the production and release of vasodilators by luteal endothelial cells (Miyamoto *et al.* 2005). These substances are necessary to trigger and initiate the luteolytic cascade, ultimately leading to the regression of the CL and the onset of luteolysis (Shirasuna *et al.* 2008). The fact that the intraluteal bloodstream in midcycle CL luteolysis, first increased at 0.5-2 hrs after PGF2α administration and the local emissions of ET-1 and Ang-II supported this theory. Moreover, ET-1 inhibits P_4 release from CL *in vitro* (Miyamoto *et al.* 1997). Furthermore, Ang-II also suppresses the local production of P_4 from midcycle corpus luteum (Hayashi and Miyamoto, 1999). The present study was limited due to relatively small sample size, which might restrict the generalizability and statistical power of the findings. Therefore, further studies involving larger and more diverse cohorts are warranted to validate these observations and enhance the robustness of the conclusions.

In conclusion, the present findings indicated that PGF2α is associated with an initial increase in intraluteal blood flow followed by a progressive decline in the midcycle CL. This transient change may contribute to the initiation of luteolysis. The study provided insights into real-time hemodynamic changes during PGF2α administration in Tharparkar (*Bos indicus*) cows, particularly during the early vascular response observed within 1 h. However, further studies are required to confirm these observations and to elucidate the underlying molecular mechanisms at different stages of CL development.

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