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Luteal haemodynamics during spontaneous and induced luteolysis in crossbred cattle

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Corpus luteum (CL) is a dynamic structure, which exhibits regular periods of growth, development and regression throughout the fertile phases of reproduction. Luteolysis is the key event in the normal luteal life span that determines the cyclicity of the animals. In cattle, luteolysis is initiated by uterine prostaglandin (PG) released at the late phase of the oestrous cycle. Programmed induction of complete luteolysisis is possible by administration of exogenous PG during the mid-luteal phase of the oestrous cycle, but the CL is refractory to PG treatment during the early-luteal phase (Beal *et al*. 1980). The non-responsiveness of early CL was suggested to be due to luteal insensitivity or insufficient numbers of PG receptors (Duchens *et al*. 1994). Since CL, the most highly vascularized structure in the body, is a site of intense angiogenesis (Gaytan *et al*. 1999), it was hypothesized that vascular characteristics of the structure plays the major role in the initial responsiveness of the CL to PG. Hence, the present work aimed at documenting the luteal haemodynamics in response to exogenous PG administration during early and mid-luteal phase of the oestrous cycle.

Six healthy and regularly cyclic Jersey crossbred pluriparous cows (5 to 6 years) in the late stage of lactation, maintained at the Centralized Embryo Biotechnology Unit, Department of Animal Biotechnology, Madras Veterinary College, Chennai, were enrolled for the study. All the cows were maintained under similar stall-fed conditions throughout the study.

Initially, as a control study, the luteal blood flow was recorded every other day throughout the oestrous cycle starting from the day of observed oestrus (Day 0) to subsequent oestrus in all the animals. After ovulation, the luteal tissue was recognized and the vascular characteristics were recorded sequentially till the end of the cycle, using colour and pulsed Doppler mode of ultrasonography (Esaote, MyLab30 Vet Gold, Italy) equipped with a 7.5-MHz trans-rectal linear transducer (Acosta *et al.* 2002, Satheshkumar 2020). Blood flow mapping of the CL was conducted in various transverse sections and the intensity of the blood flow was graded as G-1 (low), G-2 (medium), G-3 (high) and G-4 (very high), as described by Kaya *et al*. (2017).

After the control study, all the animals were subjected to PG treatment (Inj. Dinoprost tromethamine; 25 mg; i.m) during two stages of the cycle, viz. Early-luteal phase i.e. Day 4 (PGEL; n=10) and Mid-luteal phase i.e. Day 10 (PGML; n=10). Changes in the intensity of the luteal blood flow were recorded, as described previously, at 0 h (time of PG administration), 1 h, 2 h, 24 h and 48 h after PG administration. The quantitative parameters of the luteal blood flow, viz. peak systolic velocity (PSV), end diastolic velocity (EDV) and resistivity index (RI) were recorded during each ultrasonographic examination (Satheshkumar 2018). The quantitative data of velocity parameters and RI were statistically analysed by students 't' test (Snedecor and Cochran 1994).

In the untreated cycle, blood flow could be observed as sparse colour Doppler signals around the periphery of the luteal tissue (G-1) from Day 3, which increased gradually and covered more than 70% of the luteal circumference (G-2) during the mid-luteal phase. The progressive increase in luteal blood flow could be attributed to the increasing endocrine activity of the structure (Kaya *et al*. 2017). The vascularity enables the luteal cells to obtain the oxygen, nutrients and hormone precursors that are indispensable to support its endocrine activity (Fraser and Wullf 2003). There was an intense increase in blood flow (G-4) between Day 15–17 of the oestrous cycle, which could be appreciated by the penetration of colour Doppler signals deep into the luteal parenchyma (Fig. 1). Miyamoto *et al.* (2006) also recorded an increase in luteal blood flow during the similar period and correlated the same to the pulsatile release of PG from the uterus. After that stage, the intensity of blood flow signals decreased and were restricted to the periphery (G-1) coinciding with the progressive luteal regression towards the final stages of the cycle. The physiology of post-PG events involved a reduction in angiogenic activity within the CL and consequently the death of steroidogenic luteal cells and tissue degradation (Siqueira *et al*. 2019).

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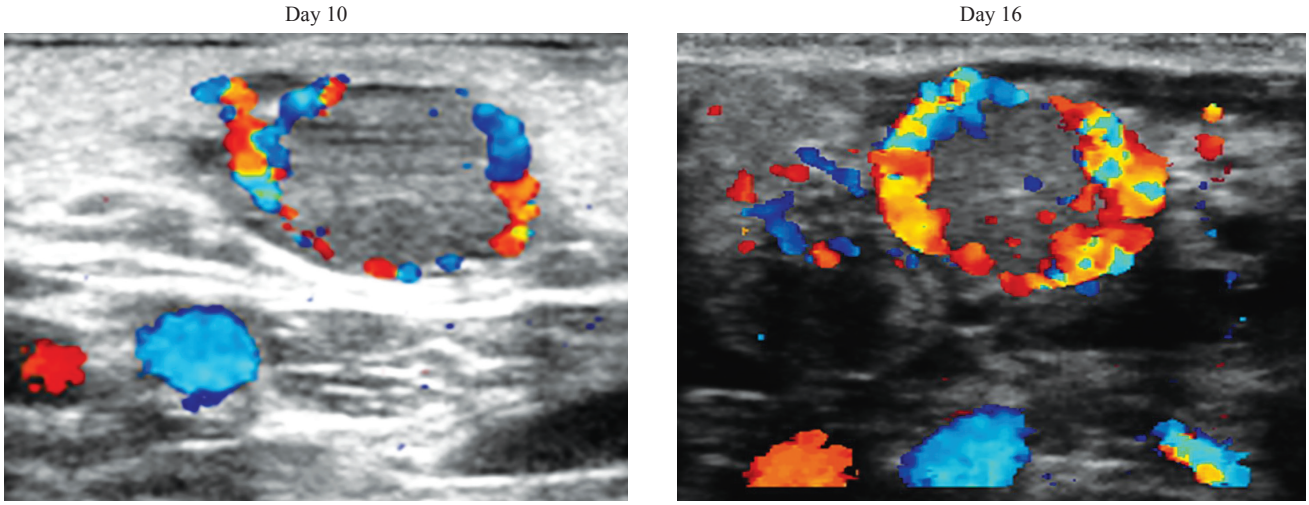

Fig. 1. Deep vascular perfusion of colour Doppler signals on Day 10 and Day 16 in untreated oestrous cycle.

Thus, it could be speculated that the increased vascular perfusion in the CL during Day 15–17 of the cycle might be the primary luteal response to endogenous PG that would have initiated the luteolytic process.

Luteal blood flow patterns in response to PG treatment in PGEL and PGML groups are represented in Fig. 2. In both the treatment groups, an increase in blood flow (G-2) was observed around the periphery of the CL 1 h post PG, as reported previously by Miyamoto and Shirasuna (2009) and Shrestha and Ginther (2011). Subsequently, 2 h post PG, the vascular intensity increased and covered more than 90% of the luteal circumference (G-3) in the PGEL group. Whereas, in the PGML group, the peripheral blood flow intensified and radiated deep into the structure spreading over 80% of the luteal parenchyma (G-4), 2 h after administration of PG. The blood flow intensity reduced and restricted to the luteal periphery (G-2), 24 h post PG, in both the groups (Pinaffi *et al*. 2018). By 48 h post PG, lysis of luteal structure was evident along with reduced blood flow intensity (G-1) in PGML group while the intensity was medium (G-2) with no signs of lysis in PGEL group. Thus, it could be concluded that PG induced variations in the intensity and nature of vascular perfusion might be the stimulating factor for initiation of luteolysis in cattle.

Investigations on luteal blood vessels revealed that microcapillary vessels were present in both the peripheral and central regions of early and mid-luteal phase CL, but large blood vessels (>20 µm) were abundant in the periphery of the mid CL when compared to the early CL (Shirasuna *et al*. 2008). Miyamoto and Shirasuna (2009) suggested that PG stimulated the endothelial nitric oxide synthase – nitric oxide (eNOS-NO) system and caused the vasodilation of peripheral blood vessels of the mid CL. Based on our observation, it can be concluded that

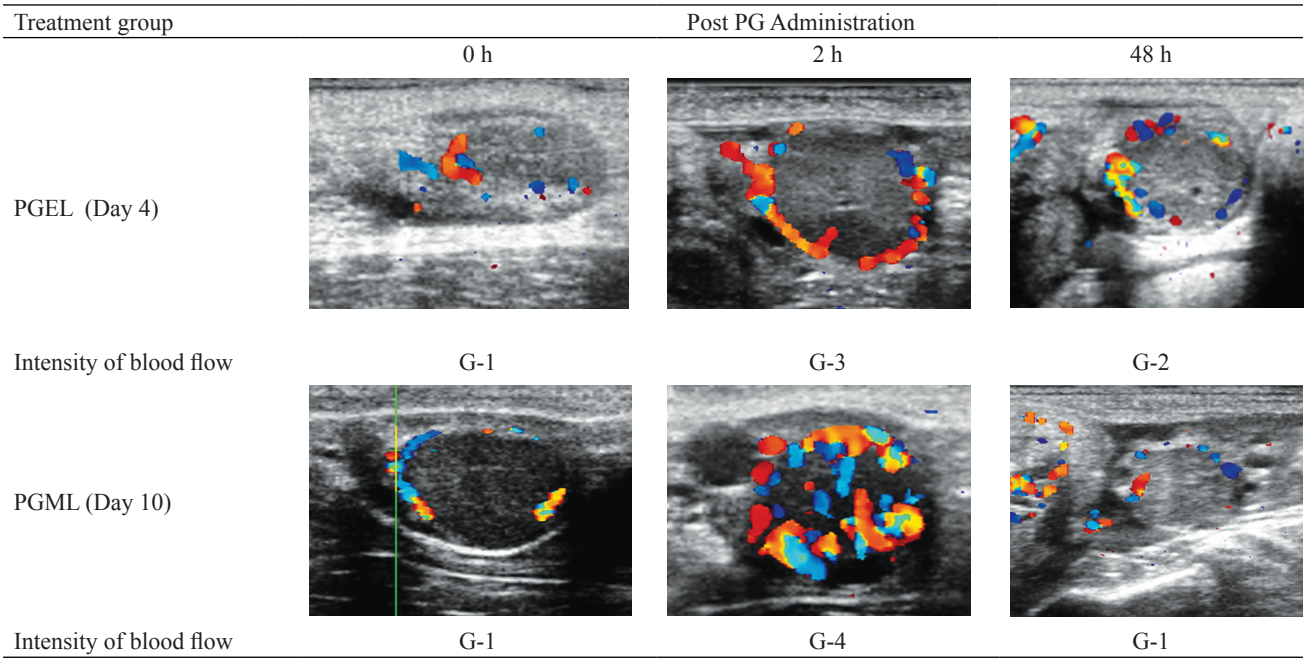

Fig. 2. Sequential changes in luteal vascular perfusion patterns after PG administration in treatment groups.

Table 1. Mean±SE of resistivity index of luteal blood flow in response to PG

Treatment group	Resistivity index (RI)				
	0 h	1 h	2 h	24 h	48 h
PGEL	0.62±0.04	0.47±0.09	0.46±0.04	0.72±0.90	0.56±0.19[a]
PGML	0.57±0.04	0.50±0.19	0.42±0.04	0.72±0.30	0.77±0.09[b]
Significance	NS	NS	NS	NS	*

*P<0.05. Values with different superscripts within the column differ significantly. NS, Not significant.

PG induced eNOS-NO system would have dilated the microvascular bed within the mature mid luteal phase CL, resulting in free flow of blood deep into the luteal parenchyma. This vascular perfusion would have enabled the luteolysin to reach the deeper luteal cells in the mature CL and initiated the lysis process.

There were no significant differences in the quantitative parameters (PSV, EDV and RI) of luteal blood flow in response to PG in both the treatment groups. However, the RI value increased significantly (P<0.05) 48 h post PG in PGML group as compared to its counterpart (Table 1), which could be attributed to the restricted blood flow in the regressing CL.

In untreated cycle, prior to spontaneous luteolysis, the intensity of blood flow increased gradually over an extended period of 2-3 days, but in treatment groups, the vascular perfusion episode was acute and rapid within hours after PG administration (Shrestha *et al*. 2010). Ginther *et al*. (2007) attributed the prolonged period of vascular perfusion in natural cycles to the pulsatile release of PGF at sequential intervals, as against the exogenous administration.

Based on the observations, it can be concluded that both spontaneous and PG induced luteolysis are characterized by an initial increase in luteal blood flow. Similarly, vascular perfusion into the parenchyma, following PG administration, enabled the luteolytic process in the mature CL of mid-luteal phase, while restricted blood flow prevented luteolytic response in early immature CL. The maturity of luteal cells along with the associated development of intra-parenchymal vasculature is warranted for the positive luteolytic response of CL. Thus, the proposed hypothesis that haemodynamics determine the luteolytic nature of the CL is proved in cattle.

SUMMARY

The haemodynamics of CL during natural and induced luteolysis was studied in crossbred cattle using colour Doppler ultrasonography. In natural cycle, penetration of Doppler signals deep into the CL was observed during Day 15–17 coinciding with the initiation of luteolysis. When PG was administered in the early luteal phase (Day 4), there was an increase in blood flow intensity in the periphery of CL, but when PG was administered in the mid luteal phase (Day 10), the intensified Doppler signals penetrated deep into the luteal parenchyma within 2 h. There were no significant differences in velocity parameters and RI values between the treatment groups. Thus, it was concluded that the characteristic difference in the intensity and nature of vascular perfusion was the determining factor for the initiation of luteolytic process in cattle.

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