

QUALITATIVE AND QUANTITATIVE ANALYSES OF LUTEAL VASCULARITY DURING FIRST TRIMESTER OF PREGNANCY IN CROSSBRED COWS

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ABSTRACT

The objective of the study was to analyze the qualitative and quantitative characteristics of luteal blood flow during the first trimester of pregnancy in crossbred cows. Sixty pregnant animals were ultrasonographically investigated during three stages of gestation viz., Stage I: 25 – 35 days; Stage II: 45 – 60 days and Stage III: 75 – 90 days. The intensity of luteal blood flow was assessed and quantitative parameters were recorded. Very high intensity of luteal vascularity was found in stage I indicative of active neo-vascularization. The values of Doppler indices were reduced in stage II when compared to stage I, indicating a lesser resistance and free flow of blood as the pregnancy advanced. Progressive increase in vascularity could be attributed to the increasing demand of the CL to meet out the endocrine activity and maintenance of pregnancy.

Keywords: Corpus Luteum, Crossbred Cows, Doppler Study, Early Pregnancy, Vascular Perfusion

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INTRODUCTION

Fertility of any mammalian species is closely related to their luteal activity. In cattle, CL is the prime source for progesterone (P₄) synthesis and hence its sustained function

needs to be secured in order to support pre-implantation embryonic development and to maintain the pregnancy (Roberts, 1971; Grana-Baumgartner *et al.*, 2020). The CL is a site of intense angiogenesis and is the most vascularized structure in the body (Acosta *et al.*, 2002). The luteal vascularity enables the luteal cells to obtain the nutrient substrates and hormone precursors that are indispensable to support P₄ biosynthesis and also to release the synthesized steroid into the general circulation (Miyazaki *et al.*, 1998; Fraser and Wulff, 2003).

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Previous studies have also ascertained that the nature of vascularity reflected the functional status of CL (Herzog *et al.*, 2010; Luttgenua *et al.*, 2011; Kaya *et al.*, 2017). Hence it was hypothesized that vascular perfusion of CL played a major role in the establishment and maintenance of pregnancy.

The present work was conducted with the aim to document the characteristics of luteal vascular perfusion during the first trimester of pregnancy in Indian crossbred cattle.

MATERIAL AND METHODS

Crossbred cows (*Bos indicus* x *B. taurus*) which were brought for diagnosis of pregnancy to the Gynaecology Unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Thanjavur District, Tamil Nadu were utilized for the study. All these animals were examined ultrasonographically (Esoate, MyLab30 Vet Gold, Italy) using a 7.5-MHz trans-rectal linear transducer and confirmed for pregnancy. The stage of pregnancy was ascertained based on the breeding history and measurement of embryo / fetal dimensions (White *et al.*, 1985). Sixty crossbred cows, in the second to fourth lactation, which were diagnosed as pregnant between 25 to 35 days were selected and subjected for further investigation. These animals were examined subsequently twice between 45 – 60 days and 75 – 90 days of gestation, targeting the first trimester of pregnancy. Thus, the investigation periods were categorized into three stages of gestation viz., Stage I: 25 – 35 days, Stage II: 45 – 60 days and Stage III: 75 – 90 days.

After confirming the stage of pregnancy, the ultrasonographic imaging was switched over to Colour Doppler mode. Transducer was placed as close as possible to the ovary and color blood flow mapping of the CL was conducted in various transverse sections (Ginther, 2007). Intensity of the luteal blood flow was graded based on the proportion of the CL area filled with colour Doppler signals and expressed as low, medium, high and very high (Satheshkumar, 2018).

Switching to Pulsed Doppler mode, spectral waveforms were obtained for at least three consecutive cardiac cycles, by placing the cursor over the brightest and consistent Doppler signal (Ginther, 2007). Velocity parameters viz., peak systolic velocity (PSV), end diastolic velocity (EDV) and mean velocity (Vmn) were manually recorded using inbuilt calipers (meters per second; m / s); Velocity indices viz., Pulsatility index (PI) and Resistance index (RI) were automatically arrived with the software of the instrument. Doppler pulse duration (DPD) i.e. the duration of a cardiac cycle (milli seconds; ms) was arrived by measuring the linear distance between two subsequent EDV points (Satheshkumar, 2018).

The mean values of these quantitative measurements were used for statistical analyses. The levels of significance between the mean values of all the luteal blood flow parameters were analyzed by one way ANOVA (Snedecor and Cochran, 1994). SPSS.10.0® software was used for analysis of data.

RESULTS AND DISCUSSION

The study analyzed the qualitative and quantitative characteristics of luteal blood flow during the first trimester of pregnancy in Indian crossbred cows (Table 1). The luteal vascular intensities during different stages of gestation are depicted in Fig 1. Visual analyses revealed that the intensity of blood flow was very high in the Stage I, covering more than 90% of the luteal circumference and even reaching the deeper areas of luteal parenchyma. The very high intensity of luteal blood flow between the days 25 to 35 of gestation was suggestive of high degree angiogenesis process in the CL, which was in concurrence with the findings of Pinaffi *et al.* (2017) in *Bos taurus* heifers. They attributed the same to the increased blood flow in the ipsilateral uterine artery and ovarian artery that supplied the ovary with CL. Late embryonic mortality usually occurs between 18 - 42 days of gestation and contributed for 3.2 – 42.7 per cent of pregnancy losses in cattle (Santos *et al.*, 2004; Pohler *et al.*, 2016). Hence the endocrine competence of CL is very crucial during this period (Starbuck *et al.*, 2004). A positive correlation between luteal blood flow and P_4 concentrations upto Day 40 of pregnancy was reported earlier by Utt *et al.* (2009). P_4 concentrations below the threshold levels will impair the pregnancy associated glycoprotein concentrations and the embryonic survival (Barbato *et al.*, 2013). Based on our findings it could be suggested that the very high intensity of luteal blood flow during the early stage of gestation might be a supportive mechanism for synthesizing and releasing optimum levels of P_4 in order to sustain the pregnancy. The intensity of blood flow was high in both the subsequent stages of II and

III, covering about 80 per cent of the luteal circumference with reduced parenchymal intrusion. The maintenance of high intensity blood flow with the advancement of pregnancy ensured the stability of luteal function.

Quantitative parameters of the luteal vascular perfusion during different stages of early gestation are represented in Table. Analysis of the data revealed that there were no significant differences in velocity parameters between the groups except for PSV which was significantly ($P < 0.01$) increased in Stage III after a non-significant decrease in Stage II. The PI and RI values reduced in Stage II when compared to Stage I, indicating a lesser resistance and free flow of blood in the CL as the pregnancy advanced, which was in corroboration with the observations of Bollwein *et al.* (2002). This could be attributed to the expansion of luteal vascular bed allowing more blood flow during the second month of gestation. However, resistance to blood flow increased significantly during the third month of gestation (Stage III) which was also reflected by significant increase in PSV. Interestingly during this period, the DPD values significantly reduced, indicative of increased pulse frequency of the blood flow (Satheshkumar, 2018). Thus, optimum blood supply to the luteal structure was ensured by frequent pumping of blood, in spite of increased resistance.

From the foregoing observations, it could be inferred that active neo-vascularisation, expanded luteal vascular bed and increased pulse frequency of luteal blood flow during the first trimester of pregnancy allowed adequate blood supply to the CL and

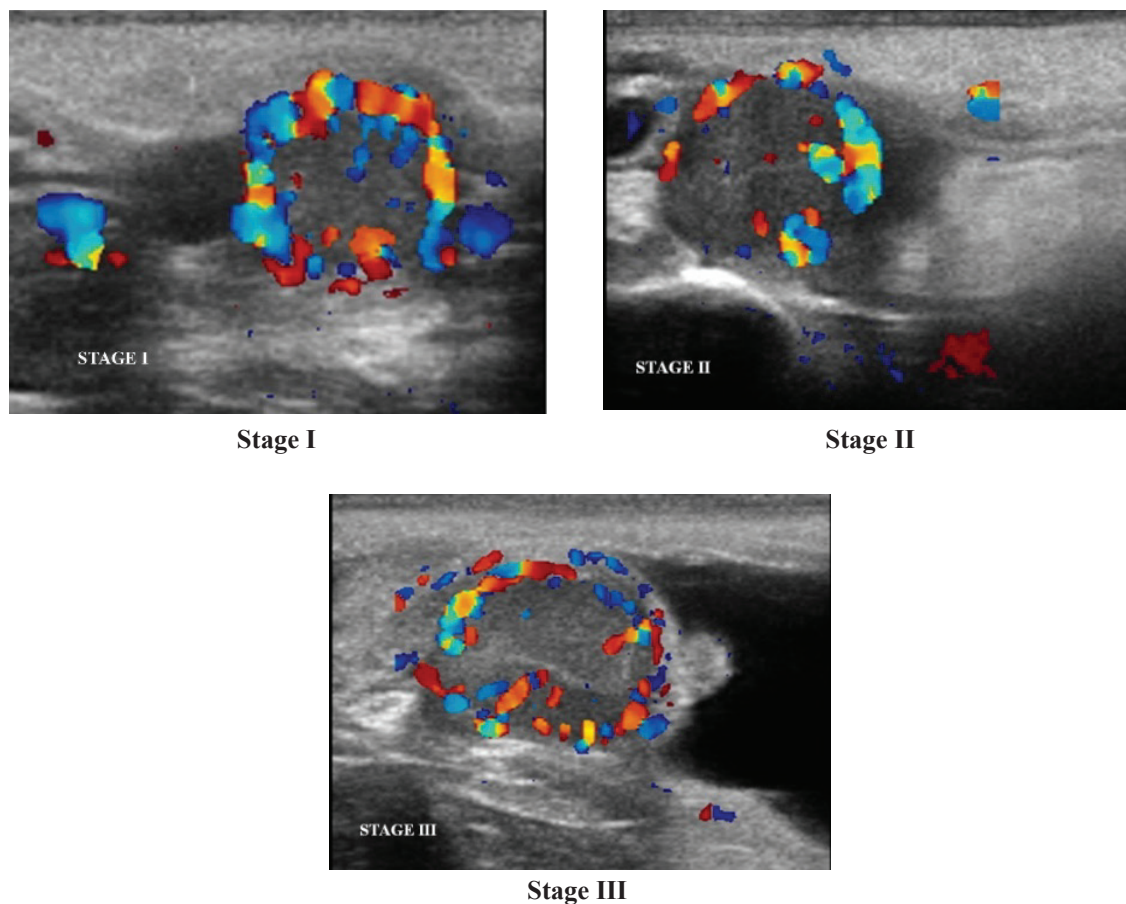


Fig. 1 Intensity of luteal blood flow during various stages of early pregnancy

Table 1. Vascular perfusion parameters of the corpus luteum during early pregnancy

Stages	PSV (m/s)	EDV (m/s)	Vmn (m/s)	PI	RI	DPD (ms)
I (25–35 days)	0.17 ^b ± 0.02	0.07 ^a ± 0.01	0.11 ^a ± 0.01	0.82 ^b ± 0.05	0.56 ^b ± 0.02	1187.33 ^b ± 48.51
II (45–60 days)	0.12 ^a ± 0.01	0.07 ^a ± 0.01	0.13 ^a ± 0.01	0.58 ^a ± 0.03	0.42 ^a ± 0.02	1180.67 ^b ± 34.10
III (75–90 days)	0.22 ^b ± 0.02	0.05 ^a ± 0.01	0.13 ^a ± 0.01	1.31 ^c ± 0.13	0.68 ^c ± 0.04	822.50 ^a ± 24.88
Significance	**	NS	NS	**	**	**

Values with different superscripts within a column differ significantly ($P < 0.01$)

** Significant ($P < 0.01$); NS- Not significant

thus supported the endocrine function and early embryonic/foetal survival.

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