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Review Article

Evaluation of bovine luteal blood flow by using color Doppler ultrasonography☆

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ABSTRACT

Since luteal vascularization plays a decisive role for the function of the corpus luteum (CL), the investigation of luteal blood flow (LBF) might give valuable information about the physiology and patho-physiology of the CL. To quantify LBF, usually Power mode color Doppler ultrasonography is used. This method detects the number of red blood cells moving through the vessels and shows them as color pixels on the B-mode image of the CL. The area of color pixels is measured with computer-assisted image analysis software and is used as a semiquantitative parameter for the assessment of LBF. Although Power mode is superior for the evaluation of LBF compared to conventional color Doppler ultrasonography, which detects the velocity of blood cells, it is still not sufficiently sensitive to detect the blood flow in the small vessels in the center of the bovine CL. Therefore, blood flow can only be measured in the bigger luteal vessels in the outer edge of the CL. Color Doppler ultrasonographic studies of the bovine estrous cycle have shown that plasma progesterone (P4) concentration can be more reliably predicted by LBF than by luteal size (LS), especially during the CL regression. During the midluteal phase, cows with low P4 level showed smaller CL, but LBF, related to LS, did not differ between cows with low and high P4 levels. In contrast to non-pregnant cows, a significant rise in LBF was observed three weeks after insemination in pregnant cows. However, LBF was not useful for an early pregnancy diagnosis due to high LBF variation among cows. When the effects of an acute systemic inflammation and exogenous hormones on the CL are examined, the LBF determination is more sensitive than LS assessment. In conclusion, color Doppler ultrasonography of the bovine CL provides additional information on luteal function compared to measurements of LS and plasma P4, but its value as a parameter concerning assessment of fertility in cows has to be clarified.

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1. Introduction

The integration of B-mode ultrasonography in bovine reproductive medicine more than 30 years ago allowed for the non-invasive evaluation of the morphology of the corpus luteum (CL) in cattle [1]. However, luteal function could be reliably assessed only by the additional determination of plasma progesterone (P4) concentration [2]. During the last 15 years, color Doppler ultrasonography has gained increasing influence in bovine reproduction and proved suitable to replace the highly invasive methods of blood flow measurement [3]. Several studies were conducted to test the benefits of this new diagnostic tool with respect to the evaluation of luteal blood flow (LBF). Therefore, cycle-associated changes of LBF, changes during early pregnancy, and alterations of LBF in response to hormonal treatments as well as systemic inflammatory conditions were determined. The present paper reviews some of the studies that provided valuable information regarding blood flow in the bovine CL and led to a better understanding of luteal development and function.

2. Measurement of luteal blood flow with color Doppler ultrasonography

The Doppler method described for human medicine by Miyazaki et al. [4] was used to quantify LBF in cows. Conventional Doppler ultrasound, like continuous-wave Doppler and pulsed-wave Doppler, is applicable to measure high blood flow velocities. Since blood vessels of the CL have a very low blood flow velocity, Power mode ultrasonography, an advanced method of color Doppler ultrasonography that detects the number of red blood cells moving through the vessel per time unit and shows them as colored pixels, is advantageous for LBF measurement [5]. The blood vessel supplying the CL splits into vessels that go around the CL (Fig. 1); therefore, blood flow appears as a colored circle around the CL. Although Power mode is superior for the evaluation of LBF, it is not sensitive enough to detect the blood flow of very small vessels in the center of the bovine CL. The area of colored pixels seen on the B-mode image in Power mode (encircled in Fig. 1) is measured with computer-assisted image analysis software (*e.g.*, PixelFlux Version 1.0; Chameleon Software, Leipzig, Germany) and is used for the semiquantitative assessment of LBF. This method has high intra-observer reproducibility. The intra-class correlation coefficient for measurements of three images recorded during the same examination period was 0.90 [6]. Color Doppler ultrasonography, therefore, seems to be a precise, non-invasive and reproducible method to evaluate the blood flow of the bovine CL.

3. Cycle-associated changes in luteal blood flow

In a recent study [7], changes in bovine LBF were determined during the whole estrous cycle by Power mode ultrasonography and were compared with the morphological changes of the CL (luteal size, LS) recorded by B-mode ultrasonography as well as with alterations in plasma P4 concentration (Fig. 2). The correlation between LBF and P4 during the estrous cycle was higher than correlations between cyclic changes in the size of the cross-sectional area of the CL (LS) and LBF or between changes in LS and P4. The close relationship between LBF and P4 seems reasonable since steroid precursors are provided to the CL *via* luteal blood vessels [8] and the release of P4 into the circulation is also dependent on adequate LBF [9].

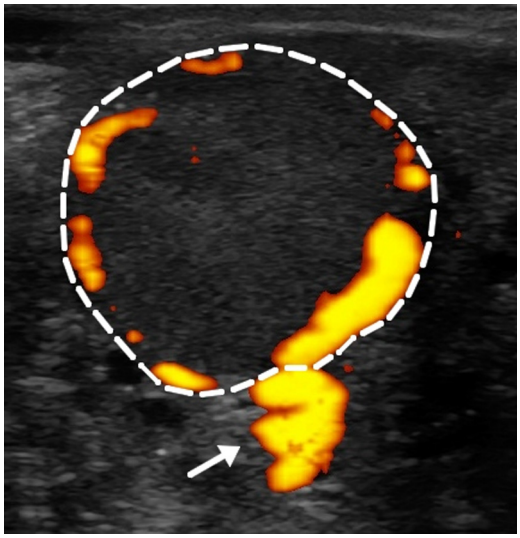

Fig. 1 – Ultrasound image of bovine corpus luteum (CL) of Day 9 of the estrous cycle obtained by Power Doppler mode. The blood vessel supplying the CL before the vessel divides into vessels enveloping the CL is also visible (arrow).

During the mid-luteal phase (Days 9–12), the relationships between luteal volume (LV) and luteal as well as plasma P4 concentrations were investigated by Lüttgenau et al. [10] (Fig. 3). A moderate positive correlation between the LV and plasma P4 ($r = 0.43$, $p < 0.05$) and a high positive correlation between LV and luteal P4 ($r = 0.70$, $p < 0.05$) were found in the study. Vasconcelos et al. [11] also observed that lower LV was associated with lower blood P4 concentration. Therefore, the quantity of luteal cells seems to be decisive for the amount of P4 produced by the bovine CL. In contrast, recent studies reported either no significant correlation [10] or a moderate positive correlation [7] between LBF and plasma P4. Since LBF and plasma P4 were both closely related to the size of the CL, positive correlations between LBF and P4 might result from LS instead from LBF. Therefore, relative LBF (rLBF; quotient of LBF and LS) was used to exclude the influence of LS on LBF [10]. There was no correlation between rLBF and plasma P4, indicating that the assessment of P4 secretion by means of blood flow is not possible in the CL of the mid-luteal phase.

Later in the mid-luteal phase (from Day 12 onwards), LBF and P4 continued to increase, whereas LS remained constant [7]. In a histological study of Lei et al. [12], an increase in the vascular space by 25% between Days 6 and 12 was observed, whereas the space of endothelial and smooth muscle cells decreased by 15%. Therefore, the observed increase in LBF