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Association of luteal blood flow with follicular size, serum estrogen and progesterone concentrations, and the inducibility of luteolysis by $PGF_{2\alpha}$ in dairy cows

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ABSTRACT

The aim of this study was to investigate the compatibility of the visual evaluation result of the blood flow characteristics and the blood flow measurements of the CL and the predictability of the responses given by corpora lutea with varying levels of blood flow to an induction of luteolysis by a $PGF_{2\alpha}$ injection and to determine the possibility of increase in serum estrogen and progesterone concentrations in parallel with increased luteal blood flow (LBF). The cows, bearing a CL ($n = 60$; postpartum 35 days), were injected with $PGF_{2\alpha}$ and were monitored for signs of estrous following the first injection. The cows, which did not show estrous signs, were examined for the presence of a CL on Day 14, whereas those that showed signs of estrous were examined on Day 10 following the onset of estrous. The level of LBF was visually graded as + (low; GI), ++ (medium; GII), +++ (high; GIII), and ++++ (very high; GIV). Immediately after the examination of LBFs, a second intramuscular injection of $PGF_{2\alpha}$ was injected. In the cows, which were determined to be in estrous, the diameter of the Graafian follicles was measured by B-mode ultrasonography. Subsequently, these animals were artificially inseminated. The animals, which did not show estrous after the second injection, were examined as previously described and monitored for signs of estrous. A strong correlation ($r = 0.654$; $P < 0.001$) was determined to exist between the results of the visual examination of the images and the results obtained for the LBF area with the use of the Pixel Flux software. GIII (0.83 ± 0.15 cm^2) and GIV (1.03 ± 0.48 cm^2) were found to differ from GI (0.47 ± 0.23 cm^2) and GII (0.51 ± 0.12 cm^2) for the size of the LBF ($P < 0.001$). Serum progesterone levels in groups (GI, GII, GIII, and GIV) were determined to be 4.44 ± 2.42 ng/mL, 6.03 ± 2.37 ng/mL, 7.01 ± 2.94 ng/mL, and 7.17 ± 1.69 ng/mL, respectively. The comparative evaluation of the study groups showed that the groups did not statistically differ for the period between $PGF_{2\alpha}$ injection and the onset of estrous, mean Graafian follicle size and estrogen levels. No direct correlation existed between these reproductive parameters and LBF.

1. Introduction

The CL, which has a lifespan of 17 to 18 days, is an endocrine structure that is involved in the establishment and maintenance of pregnancy by producing progesterone

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[1]. Among all body tissues, it is the CL, which receives the highest level of blood flow in proportion to its size [2]. The vasculature of the CL supplies the luteal tissues and enables not only the transport of the hormones and hormonal substances required for the secretion of progesterone (P4) but also the release of secreted progesterone into the systemic blood circulation [3–6]. Furthermore, luteal endothelial cells secrete various vasoactive substances, including nitric oxide (NO), endothelin-1, angiotensin II (Ang II), and prostaglandins (PGs), all of which are directly involved in the regulation of P4 secretion. Therefore, the blood vessels and endothelial cells of the CL play an important role in the functionality of the CL [1].

Color Doppler ultrasonography is a noninvasive diagnostic method used for the visual observation of the blood flow within the CL and the wall of the preovulatory follicle [7], and the evaluation of the ovarian vascular function [8]. In the past 15 years, color Doppler ultrasonography has replaced invasive techniques for the monitoring of the bovine reproductive system [9]. Recently, luteal blood flow (LBF) measurement has started to be used for the determination of the functional status of the CL [8,10–14]. In previous research, LBF has been investigated throughout the estrous cycle [15], has been used for early pregnancy diagnosis [16,17] and the detection of nonpregnant dairy and beef cows at Day 20 after timed artificial insemination [18–20], and has been tested in response to different hormone treatments [14]. Some literature reports suggest that LBF increases in parallel with the increase of the size of the CL and progesterone level during the development of the CL and indicate that LBF level is strongly correlated with progesterone production [5,15,21].

Luteolysis is described as the lysis or structural death of the CL [22]. Prostaglandin F2 alpha is a luteolytic factor, which is secreted from the uterus and initiates luteolysis in the CL. It causes a striking decrease in the progesterone level and reduces the size of the CL [1]. It has been reported that $PGF_{2\alpha}$ receptors are mostly located in the endothelial cells and large blood vessels situated in the periphery of the CL and are found to a less extent in the small blood vessels in the center of the CL. Prostaglandin F2 alpha acutely stimulates endothelial NO synthase and increases LBF in the periphery of the CL. Nitric oxide is directly involved in the regression of the CL, owing to its vasodilator effect of the arterioles [23]. Increasing the LBF, accompanied by immune cell infiltration, increase in chemokines, and expression of major histocompatibility complex molecules, causes the functional luteolysis. But then, $PGF_{2\alpha}$ directly increases endothelin-1 and Ang II secretion from microcapillary vessels within the CL. These vasoactive peptides suppress P4 secretion and induce chronic vasoconstriction of the arterioles of the CL. Structural regression of the CL is indicated by a gradual reduction in CL size and ensure luteolysis [24]. The prolongation of the luteolysis results in the prolonged dominance of the preovulatory follicle [25]. The permanence of the dominant follicle alters the environmental factors that influence oocyte development and causes embryonic degeneration, which eventually leads to reduced fertility [26]. Therefore, it is suggested that cows with higher LBF levels may display a more evident estrous response to $PGF_{2\alpha}$ injection. The aim of this study was to investigate the compatibility of the visual evaluation result of the blood flow characteristics and the blood flow measurements of the CL and to determine the responses of corpora lutea (different proportion of luteal area with blood flow signals) to $PGF_{2\alpha}$ treatment and the correlation of LBF level with follicle size and estrogen and progesterone levels.

2. Materials and methods

This study was conducted after obtaining approval from the Animal Experiments Local Ethics Committee of Selcuk University (SUVFEK—Submission: 19.02.2013/004).

2.1. Animals

The study material comprised of 60 Holstein cattle, which were raised in a semi-closed system in a private holding and fed on a total mixed ration (corn silage, dry alfalfa, dry vetch-triticale, wheat hay, corn flex, soya oilcake, and pellet feed for dairy cows). The average parity of the cows (aged 3–11 years) was 2.53 $\pm$ 0.22. Immediately after the first $PGF_{2\alpha}$ injection, body condition scores were assessed as described by Edmonson et al. [27] (scale 1–5, in increments of 0.25). Although the body condition scores of the animals ranged between 2.50 and 3.75, their average milk yield during lactation was 27.5 $\pm$ 5.5 L.

2.2. Assessment of luteal blood flow

Clinically healthy animals, which were determined to have a CL by transrectal palpation and ultrasonographic examination on Day 35 postpartum, were injected with $PGF_{2\alpha}$ (5 mL, 5 mg/mL, dinoprost, Enzaprost, CEVA-DIF, Turkey) and were monitored for signs of estrous following this first injection. The animals, which did not present with any signs of estrous, were examined on Day 14 after the first $PGF_{2\alpha}$ (5 mL, 5 mg/mL, dinoprost, Enzaprost, CEVA-DIF) injection, whereas the animals, which displayed signs of estrous, were examined on Day 10 after the onset of estrous for the presence of a CL. B-mode and power Doppler mode ultrasonographic examinations and image collection were performed as described previously [11,28]. The CL was identified by B-mode ultrasonography (10-MHz frequency, LOGIQ Book XP, General Electric Healthcare, Solingen, Germany), and its image was frozen at the maximum cross-sectional area and stored for further off-line measurements. Later, the images were examined by the power Doppler mode (gain: 19.5, pulse repetition frequency: 0.5 KHz, Doppler frequency: 5 MHz) of the same device equipped with linear probe for the imaging of the blood flow to the CL. Color LBF mapping in various transverse sections was conducted using the power Doppler mode. To minimize the variations in recording, the settings of the power Doppler system were fixed and used for all examinations. At least five images without flash artifacts and with the maximum number of colored areas were stored in the memory of the ultrasound device in Digital Imaging and Communications in Medicine (DICOM) format. The analysis of the stored Doppler images (five images of the blood flow area of the CL) was performed using an image processing software (Pixel Flux, Version 1.0,