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Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows

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Abstract

The objectives of this experiment were to characterize luteal blood flow in pregnant and non-pregnant cows and to determine its value for early pregnancy diagnosis. Lactating dairy cows (n = 54), 5.2 ± 0.2 y old (mean ± SEM), average parity 2.4 ± 0.2, and ≥ 6 wk postpartum at the start of the study, were used. The corpus luteum (CL) was examined with transrectal color Doppler ultrasonography (10.0-MHz linear-array transducer) on Days 3, 6, 9, 11, 13, 15, 18, and 21 of the estrus cycle (estrus = Day 0). Artificially inseminated cows (n = 40) were retrospectively classified as pregnant (embryonic heartbeat on Day 25; n = 18), nonpregnant (interestrus interval 15 to 21 d, n = 18), or having an apparent early embryonic loss (interestrus interval >25 d, n = 4). There was a group by time interaction ($P < 0.001$) for luteal blood flow from Days 3 to 18; it was approximately 1.10 ± 0.08 cm^2 (mean ± SEM) on Day 3, and increased to approximately 2.00 ± 0.08 cm^2 on Day 13 (similar among groups). Thereafter, luteal blood flow was numerically (albeit not significantly) greater in pregnant cows, remained constant in those with apparent embryonic loss, and declined (not significantly) between Days 15 and 18 in nonpregnant cows. Luteal blood flow was greater in pregnant than in nonpregnant ($P < 0.05$) and nonbred cows ($P < 0.05$, n = 14) on Day 15 (2.50 ± 0.16, 2.01 ± 0.16, and 2.00 ± 0.18 cm^2, respectively) and on Day 18 (2.40 ± 0.19, 1.45 ± 0.19, and 0.95 ± 0.21 cm^2). In cows with apparent early embryonic loss, luteal blood flow was 2.00 ± 0.34 and 2.05 ± 0.39 cm^2 on Days 15 and 18, which was less (not significantly) than in pregnant cows, but greater ($P < 0.05$) than in nonbred cows on Day 18. Although mean luteal blood flow was significantly greater in pregnant than nonpregnant (and nonbred) cows on Days 15 and 18, due to substantial variation among cows, it was not an appropriate diagnostic tool for pregnancy status.

Keywords: Corpus luteum; Embryonic mortality; Pregnancy diagnosis; Color Doppler ultrasonography; Cattle

1. Introduction

Embryonic loss is the most important cause of pregnancy wastage in cattle. Although up to 40% of embryonic losses occur between Days 8 and 17 of pregnancy [1], neither the underlying causes for this high rate of loss nor the complex embryo-maternal interactions during the preimplantation period have been completely elucidated [2,3]. Losses that occurred toward the end of this interval apparently involved failure to sustain the CL [1,4]. In these cattle, it was speculated that embryonic IFN_τ production and inhibition of uter-

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ine $PGF_{2\alpha}$ secretion was initiated but not sustained, resulting in prolonged interovulatory intervals [5,6]. In contrast, physiologic interovulatory intervals (comparable to those of nonbred cattle) following insemination were attributed to conception failure or embryonic loss prior to the onset of maternal recognition of pregnancy, with no extension of CL lifespan [7].

Vasoactive substances produced by the embryo, endometrium, or both, may increase blood perfusion and play a role in pregnancy recognition [8]. In a recent study which used non-invasive transrectal Doppler ultrasonography, uterine blood flow increased during the first 3 weeks of pregnancy [9]. In another study, Doppler ultrasonography was used to investigate luteal blood flow and to identify non-pregnant cattle during the first 3 weeks after embryo-transfer, based on the decreased luteal perfusion associated with luteolysis [10]. Although luteal blood flow has been investigated extensively during spontaneous and induced luteolysis [11–14], there are apparently no other reports comparing luteal perfusion in pregnant and nonpregnant cattle during this critical interval.

The first objective of the present study was to characterize luteal blood flow in pregnant, nonpregant, and nonbred cows, and cows with apparent early embryonic loss (prolonged interestrus intervals). The second objective was to determine the predictive value of luteal blood flow as a method of early pregnancy diagnosis.

2. Materials and methods

2.1. Cows

This study was conducted using the experimental herd of the Institute of Farm Animal Genetics in Mariensee, Germany between April 2005 and January 2006 and was approved and conducted in accordance with German legislation on animal welfare (Lower Saxony Federal State Office for Consumer Protection and Food Safety, 33-42502 – 06/1185). Fifty-four lactating German Holstein (n = 12), German Black Pied (n = 17), and cross-bred cows (n = 25), clinically healthy and with no apparent reproductive abnormalities, were used for this study. The animals were 5.2 ± 0.2 y old (mean ± SEM; range, 2.9 to 11.3), parity 2.4 ± 0.2 (range, 1 to 7), and were ≥6 wk postpartum. The cows were housed in tie-stalls, fed *ad libitum* (6.6 MJ NEL/kg dry matter, 14.9% crude protein) from October to May, and pastured from June to September. In addition, they were individually fed concentrates, commensurate with milk production (approximately 1 kg concentrate for each 1 kg of milk produced). Fresh water was available *ad libitum*. The cows were observed for 40 min, twice daily (morning and late afternoon) for estrous behavior. The interestrus interval was 18 to 24 d in all cows.

2.2. Study design

Cows were randomly allocated into two groups. One group (n = 40) was inseminated 12 h after first detection of standing estrus with 20×10^6 frozen/thawed sperm from one of two fertile Holstein bulls. Cows in the other group (n = 14) were not inseminated and were examined throughout one estrous cycle (nonbred group). For each cow, the CL was examined with transrectal color Doppler ultrasonography on Days 3, 6, 9, 11, 13, 15, 18, and 21 of the estrous cycle (estrus = Day 0). All cows were examined in the same sequence every day.

On Day 25, ultrasonographic pregnancy diagnosis was conducted in all inseminated cows. Cows with an embryo proper and embryonic heartbeat were designated pregnant (pregnant cows), whereas the remainder were designated as nonpregnant (nonpregnant cows) or considered to have had early embryonic loss (apparent embryonic loss cows); the latter two groups were defined on the basis of the interestrus interval (15 to 21 or >25 d, respectively).

2.3. Blood samples and determination of plasma progesterone concentrations

Immediately after each sonographic examination, blood samples were collected in evacuated blood tubes by venipuncture of coccygeal vessels, and the tubes immediately placed on ice. Plasma was separated by centrifugation (2000 × g, 20 min at 4 °C) within 1 h after collection, and stored at −20 °C until assayed. Plasma progesterone (P_4) concentrations were determined with an enzyme immunoassay [15]. Briefly, P_4 was measured directly in 20 μL plasma (monoclonal anti-P_4 antibody P-1922; Sigma, Steinheim, Germany; enzyme: 4-pregnen-3,20-dione-3-O-carboxymethyloxime horseradish peroxidase, Freising, Germany). The lower detection limit was 0.3 ng/mL and the intra- and inter-assay CV were both <10%.

2.4. Ultrasonography and determination of luteal blood flow

All sonographic investigations were conducted by the same operator, using a Logiq Book XP ultrasound device (General Electrics Medical Systems, Jiangsu, P.R. China), equipped with a 10.0-MHz linear-array transducer (General Electrics Yokogawa Medical Systems, Tokyo, Japan). Three cross-sectional images with maximal areas of the respective CL were recorded and