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Corpus luteum blood flow evaluation on Day 21 to improve the management of embryo recipient herds

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ABSTRACT

The aim of the present study was to use blood flow evaluation of the CL at 14 days after embryo transfer to detect nonpregnant animals and optimize the management of bovine recipients. The estrous cycle was synchronized in 165 recipients, and the day of expected ovulation was considered to be Day 0. Embryo transfer was performed 7 days later, on Day 7. On Day 21, pregnancy was diagnosed on the basis of blood flow evaluation of the CL (DG21—predictive diagnostic). To validate this methodology, visual scores for blood flow were compared to objective data extracted from CL ultrasound images recorded in the Doppler mode. The size was also evaluated using recorded images of the CL in the B mode. Blood samples were also collected for further analysis of the progesterone (P4) concentration. The diagnosis of pregnancy was confirmed at 35 days after estrus (DG35—definitive diagnostic). The DG21 showed that 55.2% (90 of 163) of the animals were presumptively pregnant, and this value was higher ($P < 0.04$) than that obtained at DG35 (43.6%, 71 of 163). The predictive diagnostic achieved moderate specificity (79.3%) for the detection of pregnancy, but most importantly, high sensitivity (100%) for the detection of nonpregnant recipients. The overall accuracy of the diagnosis was 88.3%. The P4 concentrations were different ($P < 0.02$) and correlated with each visual score assigned for the CL size. Visual scores for CL blood flow were also efficient ($P < 0.0001$) to distinguish animals with different levels of P4; however, P4 concentrations were higher for scores 1 and 2 (high and regular blood flow, respectively) than those for score 3 (low blood flow). This technique showed high sensitivity and facilitated the early detection of nonpregnant animals. The DG21 would allow about 79.3% of nonpregnant animals to be resynchronized 9 to 14 days earlier, when compared to conventional management based on pregnancy diagnosis at Days 30 to 35.

1. Introduction

The management of recipients is a major cost in embryo transfer (ET) programs. In well-managed herds, obtaining large numbers of pregnancies in a short period of time is desirable. Thus, the prompt identification of nonpregnant animals and the preparation of these animals for new ET are highly important for the rationale use of bovine recipients.

The diagnosis of pregnancy in B-mode ultrasonography is based on the visual identification of the gestational vesicle. The results of this technique are based on the volume of the vesicle, and high precision is achieved when the diagnostic is performed after 25 days of gestation [1].

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Currently, diagnosis based on the blood flow evaluation of the CL enables the CL from nonpregnant animals to be distinguished, after the expected period of maternal recognition, using color Doppler ultrasonography [2].

The CL is a highly vascularized gland responsible for progesterone (P4) secretion during the luteal phase of the estrous cycle and extending throughout the gestational period in cattle. The transition to the gestational CL involves the inhibition of luteolysis and the maintenance of the luteotrophic stimulus [3]. The vascularization of the gland evaluated through color Doppler ultrasonography is positively correlated with P4 secretion [4,5]. Thus, variations in blood flow observed between pregnant and nonpregnant females from 15 to 18 days after artificial insemination (AI) could be indicative of luteolysis in nonpregnant animals; however, individual effects do not ensure high reliability of the diagnosis of pregnancy performed at this time [6]. However, during the transition from one estrous cycle to the next, approximately 20 to 21 days after AI, the evaluation of the CL blood flow facilitates the precise identification of nonpregnant animals [2].

Thus, the objective of the present study was to evaluate blood flow in the CL at 14 days after ET to detect nonpregnant animals and improve the management of bovine recipients.

2. Materials and methods

This study was conducted in the south of Minas Gerais (Brazil) using 165 crossbred embryo recipients, heifers and cows (2–5 years) in good body condition score (3.5 ± 0.5, range 1–5 [7]). The recipients were maintained in an outdoor grazing system (*Brachiaria decumbens*), with free access to water and mixture supplement containing minerals and vitamins. All animals were cycling with no reproductive abnormalities detected in gynecologic exams and were previously immunized against bovine viral diarrhea, bovine herpesvirus type I, and *Leptospira*. All experimental procedures were previously approved by the Ethics for Animal Use Committee of the University of Alfenas—Unifenas (Protocol CEUA-Unifenas 23A/2012).

2.1. Synchronization protocol

The recipients were synchronized using the hormone protocol for the timed ET. The expected day of ovulation was defined as experimental Day 0. At 11 days before ovulation (Day−11), the animals received an intravaginal P4 (Prociclar, 0.75 g; Hertape Calier, Juatuba, Minas Gerais, Brazil) device and an intramuscular injection of 2-mg estradiol benzoate (Benzoate HC; Hertape Calier). Eight days later (Day−3), the intravaginal device was removed and an intramuscular injection of 150 μg of (D+) sodium cloprostenol (Veteglan Luteolícito, Hertape Calier) was administered. On Day 2, a 1-mg injection of estradiol benzoate was administered at the conclusion of the protocol.

2.2. Experimental design

Seven days after the expected ovulation (Day 7), only those animals with a good quality of CL, grade 1 or 2 assigned on the basis of the size score evaluated through transrectal palpation [8], received a cryopreserved (ethylene glycol) embryo of Angus breed. The embryos were at the same development stage (blastocyst) and grade 1 quality (Embryo Plus, Brits, South Africa). Transcervical ET was performed in the uterine horn ipsilateral to the ovary bearing a CL. Fourteen days after ET, the predictive diagnostic of nonpregnant recipients (DG21) was performed through visual assessment (the same technician evaluated the blood flow characteristics of the CL adjusted in scores). Additional scores for the size assessment of the CL were also obtained. Video records containing the entire cross section of the CL in B- and color Doppler modes were stored for subsequent image analysis and the validation of scores for blood flow and CL size. The animals were retrospectively classified into pregnant or nonpregnant groups. In addition, a blood sample was collected for further analysis of the P4 levels. On Day 35, approximately 28 days after ET or 14 days after DG21, pregnancy was diagnosed (DG35) on the basis of visual identification of the embryonic vesicle to confirm the DG21.

2.3. Score rate on the basis of size and blood flow of the CL and DG21

On Day 21, after the entire view of the CL cross section in B mode, the same technician assigned scores on the basis of size: 1 (large), 2 (averaged), or 3 (small CL). In the color Doppler mode, the same cross section of the CL was scanned, and visual scores were assigned on the basis of color signs covering the gland: 1 (high), 2 (regular), or 3 (small blood flow signs). The predefined criteria for blood flow scores included the presence of Doppler color signs at the border of the gland and within the luteal tissue. The CL assigned a score of 1 had blood flow at the border and within the luteal tissue well distributed along the cross section. A score of 2 was also associated with blood flow along the border and within the luteal tissue but restricted to particular parts of the cross section. The CL was designated a score of 3 when small signs of the blood flow were observed in the periphery and restricted to few parts of the gland.

The predictive diagnostic (DG21) of nonpregnant was designed for recipients with a score of 3 for luteal blood flow. For animals designated as “pregnant”, the CL in the same ovary as reported on Day 7 was designated with a blood flow score of 1 or 2.

2.4. Measurements of CL (ultrasound, software, and storage unit)

The same technician performed all examinations using the same ultrasonographic device (Mindray M5). In the B mode, the machine was preset to a frequency of 6.5 MHz, with 32 frames per second, gain of 72, 100% power, a 6.1-cm image depth, and a 2-cm focal point (where most of the time the ovary was positioned on the screen). In color Doppler mode, the settings were adjusted for a velocity range of 6.0 cm/s to detect blood cell movements in small vessels [9]. For this purpose, a frequency of 4.2 MHz, with