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Ultrasonography-accessed luteal size endpoint that most closely associates with circulating progesterone during the estrous cycle and early pregnancy in beef cows

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ABSTRACT

The aim was to evaluate the associations between circulating P4 concentrations, corpus luteum (CL) size (diameter, area or volume) and blood perfusion (BP) in cows. In Experiment 1, Pearson's correlations ($P < 0.05$) with P4 concentrations were observed during CL development (D8) for total area (TA; $r = 0.76$), luteal area (ACL; $r = 0.72$), total and luteal diameter (TD and DCL respectively; $r = 0.46$). During mid-late diestrus, there was a positive correlation ($P < 0.05$) only at D15 with TA and ACL ($r > 0.60$), TD, total volume (TV) and luteal volume (VCL; $r > 0.434$). During luteal regression, the correlation was only observed at D18 for ACL ($r = 0.478$) and D20 with several variables. In Experiment 2, CL weight and ACL had the greatest correlation with P4 (r > 0.6). In Experiment 3, TA and ACL were the variables that were most closely correlated with serum P4 concentrations at D7 in recipient cows. Correlation coefficients were greater for luteal measurements when there were compact compared with cavitary CLs. In Experiment 4, there was no correlation ($P > 0.05$) between P4 and any of the variables measured on D4 and D7 in recipient cows detected in estrus. On D18 to D20, all CL characteristics were correlated ($P < 0.05$) with plasma P4, and luteal BP and BP area were more closely ($P < 0.05$) correlated than ACL. In conclusion, CL perimeter area measurements had the greatest association with luteal function during CL development; whereas for BP there was a greater correlation with P4 than luteal size during luteolysis.

1. Introduction

The corpus luteum (CL) is a transient reproductive gland with the important function of producing progesterone (P4). Adequate P4 secretion by the CL regulates estrous cycle length and pregnancy maintenance in ruminants (Carter et al., 2008; Clemente et al., 2009; Forde et al., 2012). Analysis of P4 concentrations in blood is relatively expensive and requires a long process that includes blood harvest and processing, laboratory analysis and quantitative analysis (Skenandore et al., 2017). For this reason, non-invasive and real-time methods to obtain information about CL function have been developed and include ovarian ultrasonography (Kastelic et al., 1990a; Siqueira et al., 2009).

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A greater precision for detection of CL size was reported for B-mode US compared to rectal palpation (Kastelic et al., 1990a,b). Indeed, B-mode US has been used routinely to measure luteal diameter (de Tarso et al., 2016; Ishak et al., 2017), area (Ginther et al., 2007a; Berger et al., 2017; Vrisman et al., 2018), or volume (Gomez-Seco et al., 2017; Pinaffi et al., 2017). Considering that the CL is a three-dimensional structure, measuring luteal volume (VCL) may be considered the most representative measurement of this gland. The use of conventional B-mode equipment, however, only allows for a bi-dimensional image. Thus, CL volume calculation in the majority of previous studies was performed initially by obtaining the CL diameter and performing calculations after considering the CL as a perfect sphere. Using this methodology, a positive correlation between the CL volume and circulating P4 concentrations in dairy cattle has been reported (Sartori et al., 2002; Gomez et al., 2016; Ochoa et al., 2018). Another important aspect refers to the CL cavity that needs to be accounted for and subtracted from the actual luteal tissue area. Subtraction of the cavity area from the total area is proportionately more than subtracting the cavity volume from the total volume. The CL measurements, therefore, are needed that most closely correlate with circulating P4 concentrations, taking in consideration the differences between cavitary and compact corpora lutea.

Doppler US has been used recently to evaluate the luteal blood perfusion (BP) (Acosta and Miyamoto, 2004; Bollwein et al., 2012) and to estimate CL function in cows (Matsui and Miyamoto, 2009; Pugliesi et al., 2014b). Luteal BP estimated by Doppler US is associated with P4 concentrations throughout the estrous cycle (Berger et al., 2017). Interestingly, there are indications that there is a greater association between circulating P4 and BP than between circulating P4 and luteal size in cattle (Berger et al., 2017). The stage of estrous cycle evaluated in these studies, however, may affect the correlations between P4 and CL size or BP. For example, on early diestrus, the CL is already fully vascularized but still increasing in size; whereas during late diestrus or the luteolytic period, both luteal size and BP markedly decrease in 1–2 days after beginning of luteolysis (Shrestha et al., 2010; Pugliesi et al., 2012, 2014a).

Different measurements of CL size and BP are possible, but determinations have not been conducted of the US-accessed CL characteristic that most closely correlates with P4 at different stages of estrous cycle. Hence, the objectives of the present study are: 1) to determine the correlations between plasma P4 concentrations, CL size and BP during CL development, maintenance and regression in *Bos indicus* beef cows submitted to a TAI protocol; and 2) determine which US-accessed CL characteristic that most closely correlates with luteal mass at the luteogenesis phase after induced ovulation in cows. Two additional experiments were conducted to measure the association between US characteristic and circulating P4 at the time of embryo transfer in (1) beef recipient cows and (2) in cows having spontaneous ovulations. The following hypotheses were tested: 1) the correlation between P4 concentrations and US-accessed luteal size end-points are less when evaluating cavitary compared with compact CLs; 2) CL area is the US-assessed variable with the greatest correlation with circulating P4 at the time of embryo transfer in recipient cows; and 3) BP is the luteal variable for which there are the greatest correlations with P4 concentrations during the luteal regression phase in cattle.

2. Materials and methods

Animal welfare guidelines and handling procedures recommended by the São Paulo State (Brazil) law number 11.977 were strictly followed. Nelore (*Bos indicus*) and crossbreed cows with no gross reproductive anomalies were maintained in grazing conditions and received water *ad libitum* in all experiments. In Experiment 4, animals were additionally supplemented with sugar cane.

2.1. Experiment 1: correlations between US-assessed CL measurements and P4 concentrations during development, maintenance and regression of CL in pregnant and non-pregnant cows

Experiment 1 was designed to determine the correlations between, plasma P4 concentrations, CL size and blood perfusion during CL development, maintenance and regression. Estrous cycling, multiparous, non-lactating Nelore cows ($n = 22$) weighing 511.1 ± 11.7 kg (range: 385–668 kg) at the Pirassununga Campus of the University of São Paulo in Brazil, were subjected to an estrogen/P4 based TAI protocol. Stage of follicular growth and time of ovulation were synchronized with the insertion of an intravaginal P4 device (1.0 g of P4; Sincrogest; Ourofino Saúde Animal, Cravinhos, SP, Brazil) and i.m. treatments of estradiol benzoate (2 mg; Sincrodiol; Ourofino Saúde Animal) and PGF2α (500 µg; of sodium cloprostenol; Sincrocio; Ouro Fino Saúde Animal). Eight days later, the P4 device was removed, and the animals were treated im with a second PGF2α (500 µg; of sodium cloprostenol). Two days after device removal, cows were treated im with a gonadotropin-releasing hormone analogue (10 µg; of buserelin acetate; Sincroforte; Ouro Fino Saúde Animal) and were artificially inseminated by a single operator. The day of insemination was defined as Day 0.

Blood samples (10 ml) were collected from the jugular vein into evacuated tubes (BD Vacutainer® São Paulo, Brazil) containing heparin for plasma separation on Days 8, 12, 15, 18 and 20 post-TAI ($n = 22$) to quantify P4 concentrations. Blood samples were stored in ice before centrifugation.

Doppler and B-mode US were performed on Days 0 and 1, for ovulation detection and on Days 8, 12, 15, 18 and 20 post-TAI to measure CLs' characteristics. At the time of each ultrasonography exam, the dimensions (area and diameter of the CL and its fluid-filled anechoic cavity, if present), peripheral and total BP of the CL were evaluated. Total CL diameter (TD) and total CL area (TA) were obtained with the respective use of the "caliper" and "tracing" functions. From the TD and TA measured, the diameter (DCAV) and area (ACAV) of each CL cavity were subtracted to obtain, respectively, the effective luteal tissue diameter (DCL) and area (ACL). In addition, total CL volume (TV) was calculated from the formula of a sphere ($4*\pi*r^3/3$). Similarly, for CLs with a cavity, the cavity's volume (VCAV) was calculated considering it as a perfect sphere and the value was subtracted from the TV to estimate the luteal tissue volume (VCL; Fig. 1).

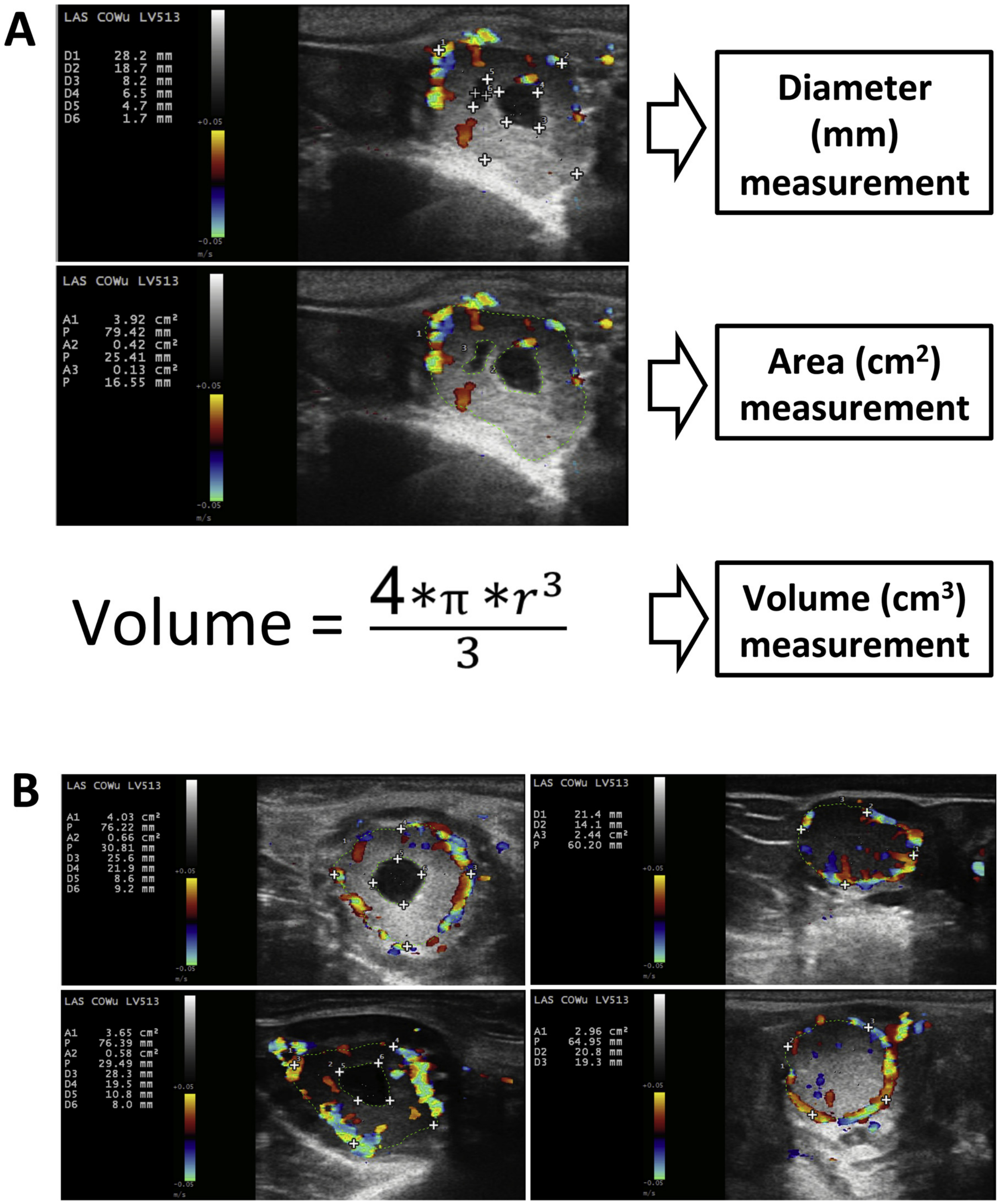

Fig. 1. Color Doppler images and formula for calculation of diameter, area and volume of total corpus luteum size or luteal tissue without a cavity (Panel A) in compact and cavitary CLs (Panel B) of cattle.

2.2. Experiment 2: correlations between CL mass and progesterone concentrations during luteogenesis in estrous cyclic cows

Because similar characteristics were observed between pregnant and non-pregnant cows during the phase of CL development (luteogenesis) in Experiment 1, non-inseminated cows were used in Experiment 2 to evaluate which US-accessed CL measurement has the greatest correlation with CL weight for P4 concentrations. Estrous cycling, multiparous, non-lactating Nelore cows (n = 22) at the research farm of the University of São Paulo in Pirassununga, SP, Brazil were submitted to a protocol to synchronize time of ovulation using the procedures described in Experiment 1. On Day 4 after ovulation, blood samples (10 ml) were collected from de the jugular vein into evacuated tubes (BD Vacutainer® São Paulo, Brazil) containing heparin for evaluation of plasma P4 concentrations. Doppler and B-mode US were performed as described previously for Experiment 1, to obtain total (TD, TA, TV) and luteal tissue (DCL, ACL and VCL) dimensions, as well as to evaluate peripheral and total BP. Within 60 min from the US exam, cows were slaughtered and reproductive tracts collected. The CLs were removed from ovaries, dissected from the ovarian stroma and weighed with the aid of an analytical precision scale. The CL weight represented the weight of the luteal mass and any luteal fluid present in the CL with cavities.

2.3. Experiment 3: correlations between US-assessed CL measurements and P4 concentrations at the time of embryo transfer in recipient cows

Based on results of Experiments 1 and 2, we aimed to determine which of ACL or total BP was most closely correlated with P4 concentration to predict CL function in commercial embryo recipients. Estrous cycling, multiparous, lactating or non-lactating crossbred ($n = 67$) and Nelore ($n = 40$) cows were subjected to a timed- embryo transfer protocol. Times of ovulation among cows were synchronized using a protocol similar to those used in Experiments 1 and 2. Briefly, cows received an intravaginal P4 device (1 g; Sincrogest), and an im treatment of estradiol benzoate (2 mg; Sincrodiol). Eight days later, the P4 device was removed, and the animals were treated im with PGF2α (500 µg; of sodium cloprostenol; SincroCio), eCG (300 IU, SincroeCG; Ourofino) and estradiol cypionate (1 mg; SincroCP; Ourofino Saúde Animal). On the day of embryo transfer (7 days after the expected ovulation; D7) a blood sample (10 ml) was collected from tail vessels into evacuated tubes (AB medical V-TUBE Gwang-Ju, South Korea) containing a serum separation gel. Immediately after blood collection, Doppler and B-mode US were performed for ACL and total BP determination as described previously for Experiment 1. Because of the time need for serum separation without centrifuging and the distance from the farm to the laboratory, the tubes were kept in at 4 °C for 48 h before serum harvest.

2.4. Experiment 4: correlations between US-assessed CL measurements and P4 concentrations during luteogenesis, maintenance and regression of CL in cows with spontaneous ovulation

In contrast to Experiments 1 to 3, only cows ($n = 50$) with spontaneous ovulations after estrous detection were used. Cows were submitted to a hormonal protocol similar to Experiments 1 and 2 and estrus was detected between 2 and 5 days after the PGF injection administered on the day of P4 device removal. Cows that expressed behavioral estrus followed by ovulation received a single *in vitro* produced embryo on Day 7 ($n = 37$). On Days 4, 7, 15, 18, 19 and 20 post-estrus blood samples (10 ml) were collected from the jugular vein into evacuated tubes (BD Vacutainer® São Paulo, Brazil) containing heparin and Doppler and B-mode US were performed for measuring ACL and total BP.

2.5. Hormone assay

Plasma (Experiments 1, 2 and 4) was removed after blood centrifugation (2700 g/30 min) and stored together with the serum obtained in Experiment 3 at -20 °C until the assay be performed. Concentrations of P4 were assayed with a solid-phase Radioimmunoassay kit (Coat-a-count; Siemens or Cat. 07–170,105; MP Biomedicals). The intra- and inter-assay CV and sensitivity for P4 among experiments ranged, respectively, from 0.8% to 3.1%, 3.1%–14.4% and 0.05 to 0.1 ng/mL.

2.6. Ultrasonic assessments

A duplex B-mode (gray scale) and pulse-wave color-Doppler ultrasonic instrument (MyLab30 Vet Gold; Esaote Healthcare, Italy) equipped with a multi-frequency linear transducer (3.5–7.5 MHz) in B mode (RES-A, gain 50%, P 74 mm, X/M, PRS 1) and Doppler mode (gain 61%, PRF 730 Hz, frequency 6.3 MHz, WF 4, PRS 3, PRC M/2) was used. Total and peripheral BP of CL was estimated according to Color Doppler signals of blood flow in the CL (Ginther, 2007b; Pugliesi et al., 2014a). Two trained evaluators (one in Experiments 1, 2 and 3 and another in Experiment 4) attributed a subjective perfusion value of CL at the moment of the exams, based on the percentage of colored signals. As the evaluation is subjective, in the first experiment films of the exams were recorded and an operator blinded to the previous evaluation re-examined the films to estimate the blood perfusion. The Pearson's correlation between the two evaluations was highly significant ($r = 0.82$; $P = 0.0001$). Area of BP was calculated multiplying ACL by total BP. Pregnancy was diagnosed in Experiments 1, 3 and 4 through B-mode US detection of a viable conceptus with presence of heartbeat on D30 post-AI or estrus.

2.7. Statistical analyses

In Experiment 1, data from proportion of CL tissue that contained a cavity on Days 8, 12, 15 and 18–20 were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). Data were analyzed using one-way ANOVA followed using the least significant difference test to determine the main effect of method of CL size measurement (diameter, area or volume) on proportion of cavity size using the PROC GLM procedure (SAS version 9.2). In each experiment, correlations between US-accessed luteal characteristics, CL weight and P4 were quantified using the Pearson correlation coefficient using the PROC CORREL procedure of the Statistical Analyses Software (SAS, version 9.2). For Experiment 1, correlations on D20 were studied separately in pregnant and non-pregnant cows, as luteolysis occurred between D15 and D20 in non-pregnant cows of this experiment. Also, in Experiment 3 correlations on D7 were studied separately in cows bearing a compact (non-cavitary) or cavitary CL. Due to the larger number of observations in Experiments 3 and 4, linear regression analyses were performed using the data analysis function of Excel software

Table 1
Proportion of cavity size in the measurements of area, diameter, and volume of cavitary corpora lutea of cows in Experiment 1.

	Days			
End-point	D8	D12	D15	D18-20
Diameter (%)	27 ± 2[a]	30 ± 4[a]	26 ± 5[a]	30 ± 4[a]
Area (%)	9 ± 1[b]	9 ± 2[b]	7 ± 2[b]	8 ± 2[b]
Volume (%)	3 ± 07[c]	3 ± 1[c]	2 ± 1[c]	3 ± 1[c]

Different superscript letters in the same column indicate differences ($P < 0.05$) between characteristics by analysis of variance.

(Microsoft, USA). A probability of $P < 0.05$ indicated that an effect was significant, and a probability of $P > 0.05$ to $P < 0.1$ indicated that there was a trend for significance.

3. Results

3.1. Experiment 1

Luteal cavities were observed on D8, D12, D15, D18 and D20 in 55%, 32%, 23%, 14% and 9% of cows, respectively. Considering total days evaluated, proportion of cavity in relation to the TD, TA and TV was, respectively, 27.2 ± 1.8%, 8.9 ± 1.0% and 3.2 ± 0.6%. When evaluated separately for each day, the cavity represented a greater ($P < 0.05$) proportion of total CL size for diameter than area and volume, and for area than volume (Table 1).

During the luteogenesis phase (D8; Fig. 2), the luteal area-related variables (TA and ACL) had the greatest correlations with P4 concentrations. The TD, BP area, VCAV, ACAV and DCAV, also were positively correlated ($P < 0.05$). There, however, was no correlation between P4 ($P > 0.1$) and total BP, peripheral BP, VCL, DCL and TV. During the luteal maintenance phase, there was no correlation ($P > 0.05$) between P4 and any variables on D12 (Fig. 2). On D15, at the end of luteal maintenance phase, the greatest correlations with P4 were detected for ACL and TA, followed by TD, TV, VCL and DCL (Fig. 2). During the luteal regression phase

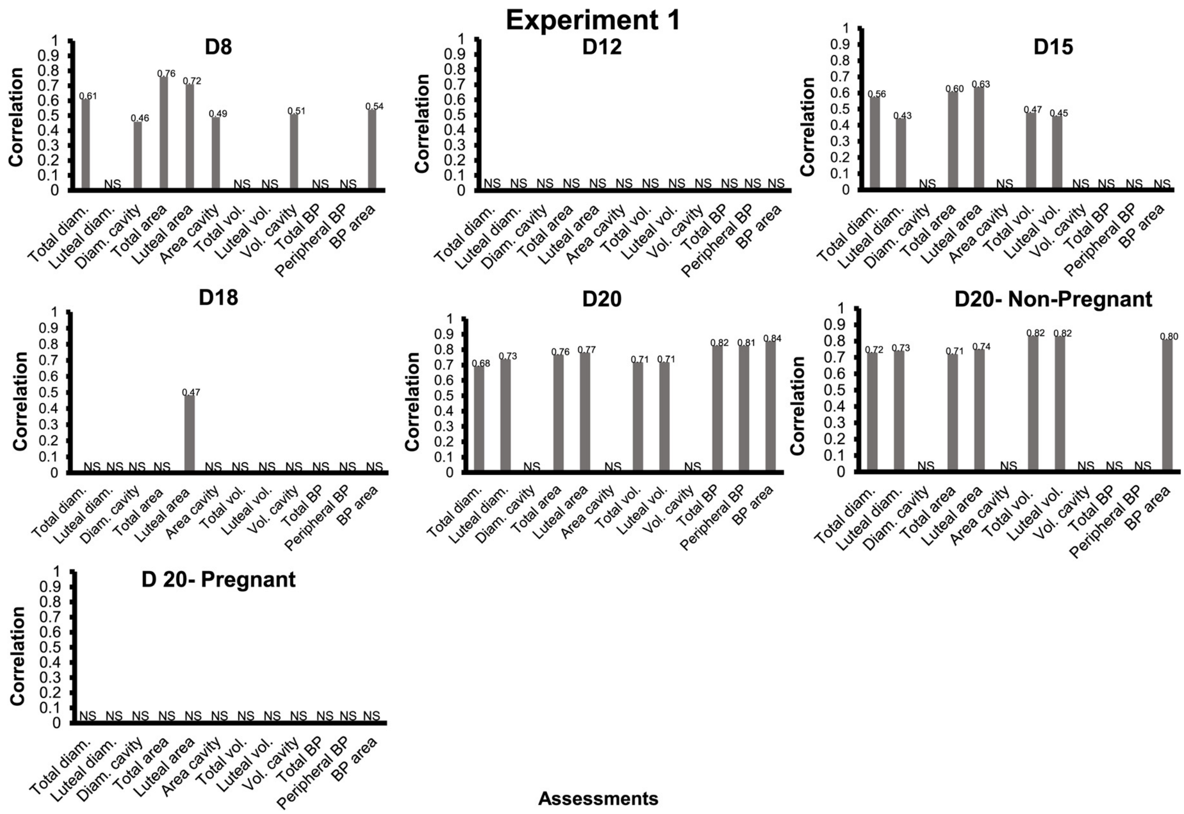

Fig. 2. Correlation coefficients (r) between plasma progesterone concentrations and ultrasonography measurements of corpus luteum during luteogenesis (day 8; D8), maintenance (Days 12 and 15; D12 and D15) and regression (Days 18 and 20; D18 and D20) phases in pregnant ($n = 10$) and non-pregnant beef cows ($n = 12$); Non-significant (NS; $P \geq 0.05$); Volume (vol); Diameter (diam); BP (blood perfusion); Experiment 1.

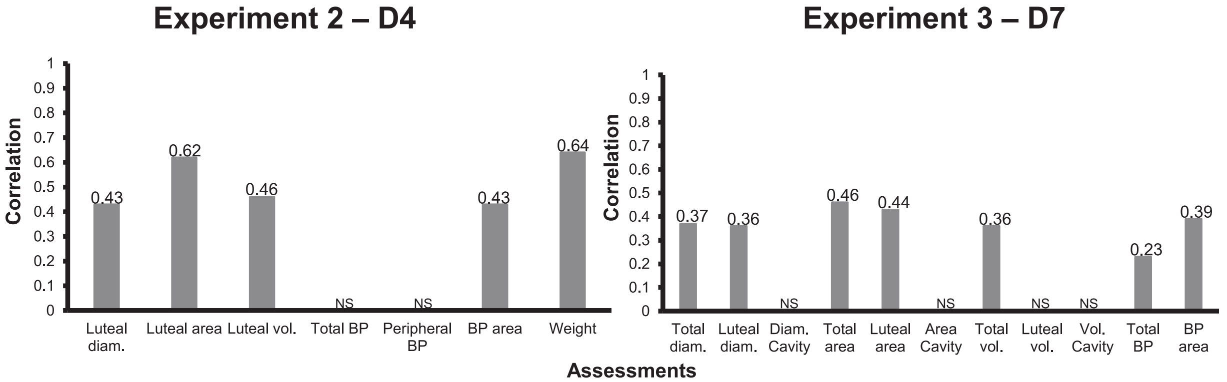

Fig. 3. Correlation coefficients (*r*) between plasma progesterone concentrations and ultrasonography measurements of corpus luteum in beef cows at luteogenesis (Day 4 and 7; D4 and D7); Non-significant (NS; P ≥ 0.05); Volume (vol); Diameter (diam); BP (blood perfusion); Experiments 2 and 3.

(D18 and D20), there was a moderate correlation ($P < 0.05$) at D18 only between P4 and ACL, and on D20, there was a strong correlation between P4 and several variables, except those related to the CL cavity. The greatest correlations measured, however, were between P4 and the BP-related variables ($r > 0.8$). When analyzed separately for the luteal regression phase (D20) for pregnant and non-pregnant cows, there were correlations ($P < 0.05$) only in non-pregnant cows.

3.2. Experiment 2

All CL size measurements were positively correlated with plasma P4 concentrations on D4. The BP assessments, however, were not correlated with P4 concentrations on D4 ($P > 0.1$). The ACL was the US-accessed CL measurement (Fig. 3) that was most closely correlated with luteal mass on D4 ($r = 0.62$; $P < 0.05$). The CL weight and ACL were the variables with the greatest correlation with plasma P4 concentrations and were very similar ($r = 0.64$ and 0.62, respectively; Fig. 3).

3.3. Experiment 3

When evaluated during the luteogenesis phase at the time of embryo transfer (D7) in recipient cows, the TA and ACL (Fig. 3) were also the variables that were most closely correlated with serum P4 concentrations, followed by BP area, TD, TV, DCL and total BP (Fig. 3). This greater association with P4 concentrations and luteal area is most precisely visualized with the linear regression analysis (Fig. 4) that indicated the positive associations between ACL and TV with serum P4 concentrations. There, however, were no correlations ($P > 0.1$) of P4 and cavity area, diameter and volume, and VCL (Fig. 3). When cows with a compact or cavitary CL were studied separately, Pearson's correlation coefficients were greater for luteal US-accessed size measurements in cows with a compact CL. There were correlations ($P < 0.0001$) with serum P4 that were moderate with total area and luteal area ($r = 0.4766$), total

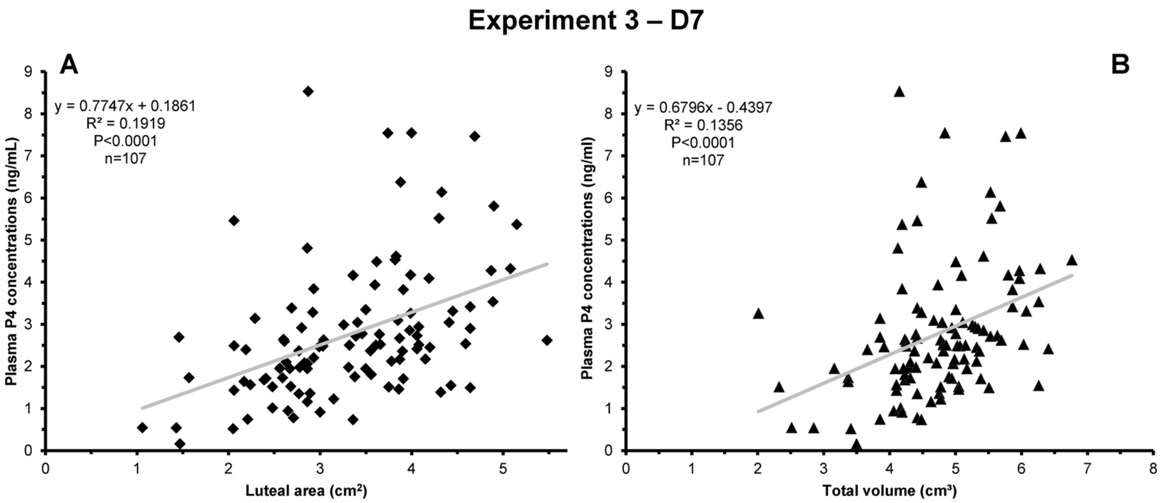

Fig. 4. Scatter plot showing the linear relationship between plasma progesterone (P4) concentrations and luteal area (ACL; left panel) or total volume (TD; right panel) in recipient cows at day of embryo transfer (7 days after expected ovulation; D7); Experiment 3.

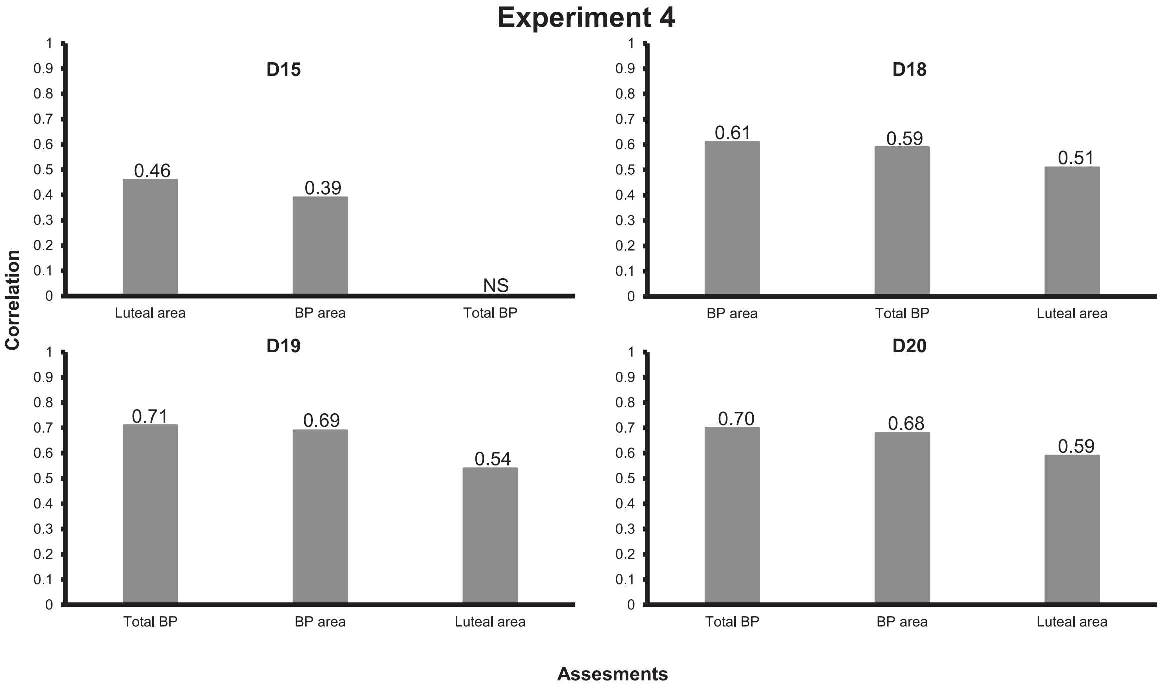

Fig. 5. Correlation coefficients (*r*) between plasma progesterone concentrations and ultrasonography measurements of corpus luteum in beef cows during maintenance (Day15; D15) and regression (Days 18, 19 and 20; D18, D19 and D20) phases; Non-significant (NS; $P \geq 0.05$); BP (blood perfusion); Experiment 4.

volume and luteal volume ($r = 0.3952$) and total diameter and luteal diameter ($r = 0.3923$) in cows with a compact CL. In cows with a cavitary CL, there were correlations ($P < 0.0001$) that were moderate to minimal for total area ($r = 0.3812$) and luteal area ($r = 0.3360$), luteal diameter ($r = 0.2354$) and total diameter ($r = 0.233$). There was no correlation of serum P4 with total volume ($r = 0.2086$; $P = 0.07$) and luteal volume ($r = 0.0589$; $P = 0.61$).

Proportion of cavity from the whole CL was greater ($P < 0.05$) when luteal tissue was evaluated as diameter (31 ± 2.0%) than area (11 ± 1%) and volume (5 ± 1%), and greater ($P < 0.05$) for area than volume.

3.4. Experiment 4

When evaluating CL from cows that expressed behavioral estrus and ovulated spontaneously, the results are different from those shown previously. During the luteogenesis phase (D4 and D7), no US-accessed variable was correlated with P4 ($P > 0.05$; data not show). During the end of the luteal maintenance phase (D15), only total BP was not correlated ($P > 0.1$) with plasma P4 concentrations. In addition, on D18, D19 and D20, all US-accessed CL characteristics were correlated ($P < 0.05$) with P4. The BP variables had the greatest correlations with plasma P4 concentrations, as indicated by the Pearson's correlation coefficients (Fig. 5) and the linear regression analyses (Fig. 6).

4. Discussion

Among the US-accessed CL size characteristics, the ACL was the one that had the greatest association with circulating P4 at different luteal phases, as consistently indicated by the greatest positive correlations in Experiments 1–3. In contrast, there were either minimal or no correlations with circulating P4 for DCL and VCL. Although moderate/high correlations between VCL and circulating P4 was reported in dairy cows (Sartori et al., 2002; Ginther, 2007a), the present results indicate that estimation of CL volume might reduce the precision of luteal size measurements. This probably occurs because CLs have non-specific shapes, which may result in an imprecise calculation of luteal volume, when considered as a perfect sphere. The ACL calculation, through measurement of the luteal perimeter results in a greater precision of estimation of luteal mass, as confirmed by the greatest and similar correlations between ACL and CL weight with P4 in Experiment 2 of the present study. These novel results, therefore, indicate that ACL is the US-accessed luteal size measurement that has the most precise association with P4 secretion throughout the estrous cycle.

In the present study, a luteal cavity was observed in more than half of the cows at early diestrus (D8) and only about 10% during the regression phase. The presence of a cavity in the CL is not considered a pathological condition because P4 secretion is not affected and about 5% of the corpora lutea during pregnancy have a central cavity (Okuda et al., 1988). In addition, the proportion of the cavity to the total CL is reduced as the estrous cycle advances in duration because the cavity reduces in size during CL development (Perez-Marin, 2009). Consistent with the present results, there were reports from an earlier study (Okuda et al., 1988) that a central

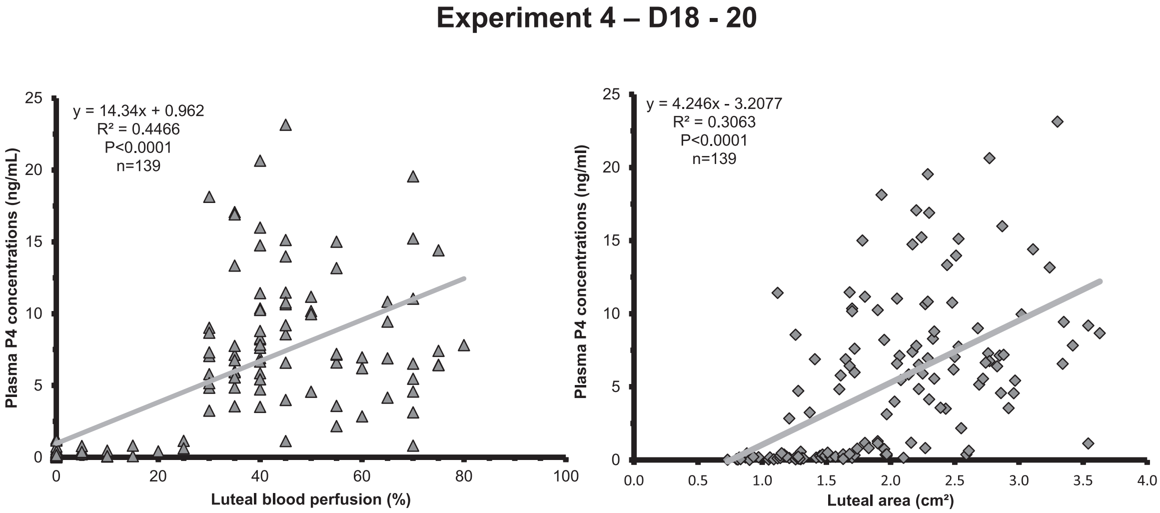

Fig. 6. Scatter plot showing the linear relationship between plasma progesterone (P4) concentrations and blood perfusion (BP; left graph) or luteal area (ACL; right graph); Experiment 4.

cavity was found in 42% (80/190) of developing corpora lutea, 34% (126/374) of fully developed corpora lutea, and 11% (7/63) in CLs during the regression period. The different proportions of CL cavity obtained when using luteal diameter, area or volume may also support the different correlations with P4 concentrations when using these different methods of measurement of luteal size. As expected, the increasing of one (diameter) to two (area) or three (volume) dimensions reduces the proportion of the cavity to the total CL tissue. The use of DCL, therefore, may increase the proportion of the cavity to total luteal size in cavitary corpora lutea and result in lesser correlations with P4. In addition, the hypothesis in the present study that the presence of a luteal cavity reduces the correlations between P4 concentrations and all ultrasonography-accessed luteal size end-points was supported by the results as indicated by the lesser correlation coefficients for all US-accessed size measurements. Presence of the cavity, however, did not affect the correlations between P4 and ACL, as indicated by only a slightly lesser correlation coefficient for cavitary compared to compact corpora lutea. The ACL, therefore, is the luteal size characteristic with the greatest correlation with circulating P4, for the evaluations of both cavitary and compact CLs.

During CL growth after an induced ovulation, luteal size was a *bona fide* indicator of luteal function, because luteal size characteristics were more precisely associated with P4 than BP characteristics. These results are consistent with those of de Tarso et al. (2017), where it was observed that there was a greater correlation between P4 and luteal area and diameter (r = 0.74 and 0.73 respectively) than P4 and BP (r = 0.42) in Aberdeen Angus cows. Also, our hypothesis that CL area is the US measurement with the greatest correlation being with circulating P4 at the time of embryo transfer in recipient cows was supported by the strong correlation value for ACL in Experiment 3 of the present study. During CL growth, the CL weight increases approximately ten times in 5 days (0.5–5 g), but this increase in size is not accompanied by a similar increase in the luteal blood flow (Robinson et al., 2007). Although evaluation of BP using Doppler ultrasonography did not result in a greater correlation with circulating P4 on D7 in the present study, results of a previous study (Pugliesi et al., 2016) indicated that luteal BP but not luteal size at the time of embryo transfer affected pregnancy rate in recipient cows. This effect reflected a progressive increase in the pregnancy rate associated with increased luteal vascularization (low, 45.1%; medium, 55.9%; and high, 62.3%). Together, these results indicate that luteal BP may indicate relevant factors of luteal activity and pregnancy maintenance other than P4 secretion by the CL.

Although synchronization of time of ovulation among cows is a practice that increased during the last two decades in TAI and TET programs (Baruselli et al., 2017), embryo transfer after estrous detection is still performed in embryo recipient operations, due to the greater pregnancy rates that are obtained. Interestingly, significant correlations with P4 at early diestrus (Experiment 4) were not observed for any US-accessed characteristic in recipient cows with spontaneous ovulation after estrous detection. Nonetheless, in previous studies there have been correlations between CL size and P4 during luteogenesis in cows (Sartori et al., 2002; Gomez-Seco et al., 2017) and buffalo (Pandey et al., 2018) with spontaneous ovulation. In this regard, cows with spontaneous ovulation generally express estrus and have a greater concentration of estradiol in the pre-ovulatory period (Rodrigues et al., 2018). The pre-ovulatory estradiol also modulates proliferation of follicular granulosa cells, which after ovulation differentiate into large luteal cells with these cells functioning to secrete 80% of the P4 produced by the CL (Donaldson and Hansel, 1965; Murdoch and Van Kirk, 1998). The lack of correlation with P4 concentrations during the CL growth phase, therefore, may be the result of the greater efficiency in P4 synthesis by cows detected in estrus (Cipriano et al., 2016). When compared to animals induced to have ovulations, cows expressing estrus have a modified pattern of gene expression in all reproductive tissues, including the CL of beef cows (Davoodi et al., 2016). In addition, the results of regression analyses (Figs. 4 and 6) are important to demonstrate the extreme variation in P4 concentrations at each measurement point. These data suggest that both after spontaneous or induced ovulations, luteal measurements may indicate active or inactive corpora lutea (Pugliesi et al., 2014a, 2018) for pregnancy diagnosis around 20–22 days of pregnancy, but are not accurate predictors of circulating concentrations of P4 during early diestrus.

When the CL reached full development (D12), no correlation with P4 concentrations was observed for any US-accessed CL measurements in the present study. In agreement, during the static phase of CL development, Herzog et al. (2010) did not observe significant correlations between P4 and CL size in dairy cows. Likewise, Balaro et al. (2017) observed only weak correlations between P4 and CL size in goats. Furthermore, there was only a weak positive correlation ($r = 0.12$) between luteal BP and P4 concentrations in dairy cows at Day 12 (Varughese et al., 2017). The absence of strong correlations during this period is probably due to the establishment of a CL final size while CL function was still increasing (Bollwein et al., 2012). In addition, fluctuations of plasma P4 concentrations are dependent on stimulus and inhibition of hormones during mid to late diestrus (Hannan et al., 2010). In this regard, P4 concentrations increased in association with the peak of an LH pulse and decreased at the peak of a prostaglandin$_{2\alpha}$ pulse (Hannan et al., 2010; Ginther et al., 2011).

During the CL regression phase, the present results indicated for the first time that both BP and luteal size are precise predictors of CL functionality, but there was a greater correlation between luteal BP and P4 concentrations than ACL and P4 concentration. The strong correlation between BP and P4 occurred on D18 to D20 in cows submitted to TAI and TET and this finding is consistent with results from other studies in cattle (Herzog et al., 2010; Lüttgenau and Bollwein, 2014). This may be attributed to the more rapid reduction in BP (65%) than in ACL (25%) at the 12 h before the end of the luteolytic period, that is closely associated with the dynamics of decreasing P4 concentrations (Pugliesi et al., 2012). An adequate supply of blood to the CL represents an important aspect for P4 secretion (Acosta and Miyamoto, 2004; Herzog et al., 2010). In goats (Balaro et al., 2017) there have been consistent findings with these previous results where BP was the most informative variable related to CL functionality during the regression phase, but CL size was the more informative during the growth phase.

5. Conclusions

In conclusion, measurement of DCL or calculating VCL as a sphere are less precise for estimating the circulating P4 concentrations than the ACL measurement by using the “tracing” function of B-mode US to determine the luteal perimeter. Also, the ACL is the CL measurement that most strongly correlates with P4 concentrations during CL development (D4 to D8); whereas correlation between P4 concentration and luteal BP was greater than luteal size end-points during the luteal regression phase. The presence of a CL cavity is observed less during mid and late luteal phases of the estrous cycle. Correlations between circulating P4 and CL measurements are less when there are cavitary than compact corpora lutea.

Conflicts of interest

None.

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References

Acosta, T.J., Miyamoto, A., 2004. Vascular control of ovarian function: ovulation, corpus luteum formation and regression. Anim. Reprod. Sci. 82–83, 127–140.
Balaro, M.F.A., Santos, A.S., Moura, L., Fonseca, J.F., Brandao, F.Z., 2017. Luteal dynamic and functionality assessment in dairy goats by luteal blood flow, luteal biometry, and hormonal assay. Theriogenology 95, 118–126.
Baruselli, P.S., Ferreira, R.M., Colli, M.H.A., Elliff, F.M., Sa, M.F., Vieira, L., de Freitas, B.G., 2017. Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. Anim. Reprod. 14, 558–571.
Berger, H., Lietzau, M., Tichy, A., Herzog, K., 2017. Pregnancy outcome is influenced by luteal area during diestrus before successful insemination but not by milk production level. Theriogenology 104, 115–119.
Bollwein, H., Lüttgenau, J., Herzog, K., 2012. Bovine luteal blood flow: basic mechanism and clinical relevance. Reprod. Fertil. Dev. 25, 71–79.
Carter, F., Forde, N., Duffy, P., Wade, M., Fair, T., Crowe, M.A., Evans, A.C.O., Kenny, D.A., Roche, J.F., Lonergan, P., 2008. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reprod. Fertil. Dev. 20, 368–375.
Cipriano, R.S., Cooke, R.F., Rodrigues, A.D., Silva, L.G.T., Bohnert, D.W., Marques, R.S., Vasconcelos, J.L.M., Pires, A.V., Cerri, R.L.A., 2016. Post-artificial insemination supplementation with calcium salts of soybean oil influences pregnancy establishment factors in Bos indicus beef cows. J. Anim. Sci. 94, 4892–4902.
Clemente, M., de la Fuente, J., Fair, T., Al Naib, A., Gutierrez-Adan, A., Roche, J.F., Rizos, D., Lonergan, P., 2009. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? Reproduction 138, 507–517.
Davoodi, S., Cooke, R.F., Fernandes, A.C.C., Cappellozza, B.I., Vasconcelos, J.L.M., Cerri, R.L.A., 2016. Expression of estrus modifies the gene expression profile in reproductive tissues on day 19 of gestation in beef cows. Theriogenology 85, 645–655.
de Tarso, S.G.S., Apgar, G.A., Gastal, M.O., Gastal, E.L., 2016. Relationships between follicle and corpus luteum diameter, blood flow, and progesterone production in beef cows and heifers: preliminary results. Anim. Reprod. 13, 81–92.
de Tarso, S.G.S., Gastal, G.D.A., Bashir, S.T., Gastal, M.O., Apgar, G.A., Gastal, E.L., 2017. Follicle vascularity coordinates corpus luteum blood flow and progesterone production. Reprod. Fertil. Dev. 29, 448–457.
Donaldson, L., Hansel, W., 1965. Histological study of bovine corpora lutea. J. Dairy Sci. 48, 905.
Forde, N., Mehta, J.P., Minten, M., Crowe, M.A., Roche, J.F., Spencer, T.E., Lonergan, P., 2012. Effects of low progesterone on the endometrial transcriptome in cattle. Biol. Reprod. 87, 11.
Ginther, O.J., 2007b. Color-Doppler Ultrasonography. 2007. Equiservices Publishing, Cross Palins, WI.
Ginther, O.J., Gastal, E.L., Gastal, M.O., Siddiqui, M.A.R., Beg, M.A., 2007. Relationships of follicle versus oocyte maturity to ultrasound morphology, blood flow, and hormone concentrations of the preovulatory follicle in mares. Biol. Reprod. 77, 202–208.
Ginther, O.J., Fuenzalida, M.J., Shrestha, H.K., Beg, M.A., 2011. Concomitance of luteinizing hormone and progesterone oscillations during the transition from

preluteolysis to luteolysis in cattle. Domest. Anim. Endocrinol. 40, 77–86.
Gomez, C., Alegre, B., Martinez-Pastor, F., Prieto, J., Gonzalez, J., Alonso, M., Dominguez, J., 2016. Evolution of the corpus luteum volume and its relation to the plasmatic progesterone concentration after artificial insemination in pregnant and non-pregnant dairy cows. Reprod. Domest. Anim. 51 95–95.
Gomez-Seco, C., Alegre, B., Martinez-Pastor, F., Prieto, J.G., Gonzalez-Montana, J.R., Alonso, M.E., Dominguez, J.C., 2017. Evolution of the corpus luteum volume determined ultrasonographically and its relation to the plasma progesterone concentration after artificial insemination in pregnant and non-pregnant dairy cows. Vet. Resear. Communi. 41, 183–188.
Hannan, M.A., Fuenzalida, M.J., Siddiqui, M.A.R., Shamsuddin, M., Beg, M.A., Ginther, O.J., 2010. Diurnal variation in LH and temporal relationships between oscillations in LH and progesterone during the luteal phase in heifers. Theriogenology 74, 1491–1498.
Herzog, K., Brockhan-Lüdemann, M., Kaske, M., Beindorff, N., Paul, V., Niemann, H., Bollwein, H., 2010. Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. Theriogenology 73, 691–697.
Ishak, G.M., Bashir, S.T., Gastal, M.O., Gastal, E.L., 2017. Pre-ovulatory follicle affects corpus luteum diameter, blood flow, and progesterone production in mares. Anim. Reprod. Sci. 187, 1–12.
Kastelic, J.P., Bergfelt, D.R., Ginther, O.J., 1990a. Relationship between ultrasonic assessment of the corpus-luteum and plasma progesterone concentration in heifers. Theriogenology 33, 1269–1278.
Kastelic, J.P., Pierson, R.A., Ginther, O.J., 1990b. Ultrasonic morphology of corpora-lutea and central luteal cavities during the estrous-cycle and early-pregnancy in heifers. Theriogenology 34, 487–498.
Lüttgenau, J., Bollwein, H., 2014. Evaluation of bovine luteal blood flow by using color Doppler ultrasonography. Reprod. Biol. 14, 103–109.
Matsui, M., Miyamoto, A., 2009. Evaluation of ovarian blood flow by colour Doppler ultrasound: practical use for reproductive management in the cow. Vet. J. 181, 232–240.
Murdoch, W.J., Van Kirk, E.A., 1998. Luteal dysfunction in ewes induced to ovulate early the follicular phase. Endocrinology 139, 3480–3484.
Ochoa, J.C., Penagaricano, F., Baez, G.M., Melo, L.F., Motta, J.C.L., Garcia-Guerra, A., Meidan, R., Ferreira, J.C.P., Sartori, R., Wiltbank, M.C., 2018. Mechanisms for rescue of corpus luteum during pregnancy: gene expression in bovine corpus luteum following intrauterine pulses of prostaglandins E-1 and F-2 alpha. Biol. Reprod. 98, 465–479.
Okuda, K., Kito, S., Sumi, N., Sato, K., 1988. A study of the central cavity in the bovine corpus-luteum. Vet. Rec. 123, 180–183.
Pandey, A.K., Ghuman, S.P.S., Dhaliwal, G.S., Honparkhe, M., Phogat, J.B., Kumar, S., 2018. Effects of preovulatory follicle size on estradiol concentrations, corpus luteum diameter, progesterone concentrations and subsequent pregnancy rate in buffalo cows (Bubalus bubalis). Theriogenology 107, 57–62.
Perez-Marin, C., 2009. Formation of corpora lutea and central luteal cavities and their relationship with plasma progesterone levels and other metabolic parameters in dairy cattle. Reprod. Domest. Anim. 44, 384–389.
Pinaffi, F.L.V., Araujo, E.R., Silva, L.A., Ginther, O.J., 2017. Color-Doppler signals of blood flow in the corpus luteum and vascular perfusion index for ovarian and uterine arteries during expansion of the allantochorion in Bos taurus heifers. Theriogenology 102, 35–43.
Pugliesi, G., Pinaffi, F.L.V., Beg, M.A., Ginther, O.J., 2012. Use of corpus luteum area as a predictor of ongoing functional luteolysis in dairy heifers IETS 2013 abstracts. Reprod. Fertil. Dev. 25, 148–316.
Pugliesi, G., Miagawa, B.T., Paiva, Y.N., França, M.R., Silva, L.A., Binelli, M., 2014a. Conceptus-induced changes in the gene expression of blood immune cells and the ultrasound-accessed luteal function in beef cattle: how early can we detect pregnancy? Biol. Reprod. 12, 91–95.
Pugliesi, G., Oliveria, M.L., Scolari, S.C., Lopes, E., Pinaffi, F.V., Miagawa, B.T., Paiva, Y.N., Maio, J.R.G., Nogueira, G.P., Binelli, M., 2014b. Corpus luteum development and function after supplementation of long-acting progesterone during the early luteal phase in beef cattle. Reprod. Domest. Anim. 49, 85–91.
Pugliesi, G., Silva, J.C.Bd., Nishimura, T.K., Miyai, D.K.C., Silva, L.A., Binelli, M., 2016. Use of Color-Doppler ultrasonography to improve selection of higher fertility beef recipient cows for embryo transfer. SBTE 2016 abstracts. Anim. Reprod. 13, 453.
Pugliesi, G., de Melo, G.D., Ataide, G.A., Pellegrino, C.A.G., Silva, J.B., Rocha, C.C., Motta, I.G., Vasconcelos, J.L.M., Binelli, M., 2018. Use of Doppler ultrasonography in embryo transfer programs: feasibility and field results. Anim. Reprod. 15, 239–246.
Robinson, R.S., Nicklin, L.T., Hammond, A.J., Schams, D., Hunter, M.G., Mann, G.E., 2007. Fibroblast growth factor 2 Is more dynamic than vascular endothelial growth factor a during the follicle-luteal transition in the cow. Biol. Reprod. 77, 28–36.
Rodrigues, A.D., Cooke, R.F., Cipriano, R.S., Silva, L.G.T., Cerri, R.L.A., Cruppe, L.H., Meneghetti, M., Pohler, K.G., Vasconcelos, J.L.M., 2018. Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in &ITBos indicus&IT-influenced beef cows. J. Anim. Sci. 96, 236–249.
Sartori, R., Rosa, G.J.M., Wiltbank, M.C., 2002. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. 85, 2813–2822.
Shrestha, H.K., Beg, M.A., Imam, S., Ginther, O.J., 2010. Luteal blood flow and concentrations of circulating progesterone and other hormones associated with a simulated pulse of 13,14-dihydro-15-keto-prostaglandin F-2 alpha in heifers. Reproduction 139, 673–683.
Siqueira, L.G.B., Torres, C.A.A., Amorim, L.S., Souza, E.D., Camargo, L.S.A., Fernandes, C.A.C., Viana, J.H.M., 2009. Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle. Anim. Reprod. Sci. 115, 18–28.
Skenandore, C.S., Pineda, A., Bahr, J.M., Newell-Fugate, A.E., Cardoso, F.C., 2017. Evaluation of a commercially available radioimmunoassay and enzyme immunoassay for the analysis of progesterone and estradiol and the comparison of two extraction efficiency methods. Domest. Anim. Endocrinol. 60, 61–66.
Varughese, E.E., Brar, P.S., Ghuman, S.S., 2017. Vascularization to preovulatory follicle and corpus luteum-a valuable predictor of fertility in dairy cows. Theriogenology 103, 59–68.
Vrisman, D.P., Bastos, N.M., Rossi, G.F., Rodrigues, N.N., Borges, L.P.B., Taira, A.R., de Paz, C.C.P., Nogueira, G.D., Teixeira, P.P.M., Monteiro, F.M., Oliveira, M.E.F., 2018. Corpus luteum dynamics after ovulation induction with or without previous exposure to progesterone in prepubertal Nellore heifers. Theriogenology 106, 60–68.