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Pregnancy rates and corpus luteum–related factors affecting pregnancy establishment in bovine recipients synchronized for fixed-time embryo transfer

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Abstract

The objective was to investigate the influence of corpora lutea physical and functional characteristics on pregnancy rates in bovine recipients synchronized for fixed-time embryo transfer (FTET). Crossbred (*Bos taurus taurus* × *Bos taurus indicus*) nonlactating cows and heifers (n = 259) were treated with the following protocol: 2 mg estradiol benzoate (EB) plus an intravaginal progesterone device (CIDR 1.9 g progesterone; Day 0); 400 IU equine chorionic gonadotropin (eCG; Day 5); prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and CIDR withdrawal (Day 8); and 1 mg EB (Day 9). Ovarian ultrasonography and blood sample collections were performed on Day 17. Of the 259 cattle initially treated, 197 (76.1%) were suitable recipients; they received a single, fresh, quality grade 1 or 2 in vivo–derived (n = 90) or in vitro–produced (n = 87) embryo on Day 17. Pregnancy rates (23 d after embryo transfer) were higher for in vivo–derived embryos than for in vitro–produced embryos (58.8% vs. 31.0%, respectively; $P < 0.001$). Mean (±SD) plasma progesterone (P_4) concentration was higher in cattle that became pregnant than that in nonpregnant cattle (5.2 ± 5.0 vs. 3.8 ± 2.4 ng/mL; $P = 0.02$). Mean pixel values (71.8 ± 1.3 vs. 71.2 ± 1.1) and pixel heterogeneity (14.8 ± 0.3 vs. 14.5 ± 0.5) were similar between pregnant and nonpregnant recipients ($P > 0.10$). No significant relationship was detected between pregnancy outcome and plasma P_4, corpus luteum area, or corpus luteum echotexture. Embryo type, however, affected the odds of pregnancy. In conclusion, corpus luteum–related traits were poor predictors of pregnancy in recipients. The type of embryo, however, was a major factor affecting pregnancy outcome.

Keywords: Cattle; Corpus luteum; Echotexture; Fixed-time embryo transfer; Ultrasonography

1. Introduction

Optimal management of bovine embryo recipients is essential in the achievement of success in an embryo transfer (ET) program [1–3]. Because recipient maintenance represents the greatest economic costs in an ET program [2], the higher the proportion of pregnant animals after ET, the greater the profit. Likewise, efficient selection and management of recipients contribute to high pregnancy rates after ET [3]. In that regard, efforts have been directed to the development of more efficient estrus synchronization protocols using a

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combination of exogenous hormones such as equine chorionic gonadotropin and estradiol benzoate [4–6] or additional treatments with specific hormones (e.g., human chorionic gonadotropin [7–10]; gonadotropin-releasing hormone analogue [11,12]) in an attempt to improve embryo survival, luteal function, and, consequently, pregnancy rates.

The standard protocol for synchronization of recipients is usually based on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [1,3]. Although $PGF_{2\alpha}$ is an effective and low-cost treatment, success depends upon estrus detection efficiency, which may be a problem on some farms. Furthermore, the corpus luteum (CL) is not responsive to $PGF_{2\alpha}$ until Day 5 of the estrous cycle, increases responsiveness up to Day 9–10, and then decreases after Day 16, resulting in a considerable asynchrony, accentuated by differences in follicle wave status among recipients [13–15]. In the case of in vitro–produced embryos, the demand for a large number of synchronized recipients is even greater, as the majority of embryos are transferred as fresh [16]. In an attempt to improve estrus synchronization and increase the number of recipients suitable to receive fresh in vitro–produced embryos, protocols based on exogenous progesterone, equine chorionic gonadotropin, and estradiol benzoate were developed and have been used successfully in large ET programs [4–6,17].

The relationships among CL size, progesterone concentrations, and pregnancy rates in recipients are still unclear and the subject of several studies [18,19]. There is disagreement about whether or not high (>6.0 ng/mL) or low (<2.0 ng/mL) circulating progesterone concentrations affect pregnancy rates [6,20–22]. Some reported that suitability of a recipient can be determined by estrus detection and the presence of a palpable CL at the time of ET, regardless of the size or quality of the CL and progesterone concentrations, as satisfactory pregnancy rates have been reported in recipients with very low (<1 ng/mL) or very high (>16 ng/mL) systemic progesterone concentrations [23]. Apparently, it is still difficult to determine whether or not circulating progesterone concentrations are useful predictors of pregnancy rates. Perhaps the explanation lies in the fact that a minimum threshold of blood progesterone concentration necessary for pregnancy in cattle is not known [24].

Corpora lutea characteristics assessed either by transrectal palpation or ultrasonography prior to ET have been used to determine which animals are suitable to receive an embryo [6,20,21,23]. By using a B-mode ultrasound scanner, not only the size but also CL echotexture can be assessed and objectively quantified [25–27]. Furthermore, CL echotexture, determined by computer-assisted analysis, seems to be a good indicator of physiologic functional status [26–30].

A practical application of this kind of technology could be evaluation of luteal function prior to ET in bovine recipients [31]. Measurement of plasma progesterone concentration on the day of ET (Day 7 ± 1) could be used to reject recipients with abnormal luteal function [32], but it is costly and time consuming. The exciting possibility of estimating luteal function based on image analysis could increase the efficiency of recipient selection and, potentially, improve pregnancy rates. To our knowledge, image analysis of the CL has not been used for this purpose. This technique may also help to clarify the relationship among CL characteristics, progesterone concentrations, and pregnancy rate.

The objective of this study was to investigate factors related to luteal function (CL size, echotexture, and plasma progesterone concentrations) that may influence the establishment of pregnancy in bovine recipients synchronized for fixed-time ET.

2. Materials and methods

2.1. Location, cattle, and experimental design

The experiment was done at eight different farms located at the former Atlantic Forest area, which covers parts of the Minas Gerais and Rio de Janeiro States in the southeast region of Brazil. Crossbred (*Bos taurus taurus* × *Bos taurus indicus*) nonlactating cows and heifers (n = 259), examined by ultrasonography to ensure evidence of ovarian cyclicity (follicular growth activity and presence of a CL), weighing 407.4 ± 67.9 kg and with body condition scores ranging from 2.5 to 3.5 (scale, 0 to 5; [33]), underwent an ovulation synchronization treatment for fixed-time embryo transfer (FTET) at unknown/random stages of the estrous cycle (Day 0). These cattle were maintained under pasture conditions (*Brachiaria* sp.), with ad libitum access to water, salt, and a mineral mixture. All eight herds enrolled in the study were under a similar management system.

The commercial products used in the synchronization protocol were estradiol benzoate (EB; Ric-BE Syntex, Buenos Aires, Argentina); controlled internal drug releasing insert (CIDR 1.9 g progesterone; Pfizer Ltda, Guarulhos, SP, Brazil); equine chorionic gonadotropin (eCG; Novormon; Syntex, Buenos Aires, Argentina), and a $PGF_{2\alpha}$ analogue (sodium cloprostenol; Ciosin; Schering-Plough Animal Health Ind. Com. Ltda, Cotia, SP, Brazil). The protocol consisted of EB treatment (2 mg, im) and CIDR insertion on Day 0; eCG

treatment (400 IU, im) on Day 5; sodium cloprostenol treatment (0.5 mg, im) and CIDR withdrawal on Day 8; and a second EB treatment (1 mg, im) on Day 9. Day 10 was arbitrarily considered the day of estrus and was followed by FTET on Day 17.

Recipients were examined ultrasonographically, and blood samples were collected for progesterone assays prior to ET. Suitability of a recipient was defined based on the presence of at least one CL; only suitable recipients received an embryo. Recipients without a CL were not used for FTET and were referred to as nonsuitable animals. Pregnancy diagnoses were performed 23 d after FTET (30 d of gestation). Corpora lutea and progesterone data were retrospectively analyzed for recipients diagnosed as pregnant or not pregnant.

2.2. Ultrasonography and image analysis

Ovaries of all recipients were scanned ultrasonically on Day 17 of the protocol, approximately 6 h before ET. Ultrasound examinations were performed using a B-mode, real-time ultrasound scanner (Aloka SSD 500; Aloka Co., Tokyo, Japan) equipped with a 5-MHz linear-array rectal transducer. All examinations were performed by the same person, and the ultrasound machine settings (near- and far-field gain, total gain, and focus position) were standardized and identical at all locations.

Corpora lutea images from each recipient were frozen on the screen to measure luteal tissue area (cm^2) using internal callipers of the ultrasound machine and recorded on VHS tapes using a videocassette recorder (VCR; 7 Head Hi-Fi Stereo HT-GSV870; Gradiente, Sao Paulo, Brazil) connected to the ultrasound by a coaxial cable. Later, recorded images were digitized to a personal computer (Pentium 4, 2.7-GHz; 1.0-GB RAM) equipped with a video capture board (Pinnacle DC10; Pinnacle Studio DC10 AV/DV, Version 9; Pinnacle Systems, Inc., Mountain View, CA, USA). Digital images were saved in 256 shades of gray as noncompressed pictures (TIFF format) and stored on the hard drive prior to image analysis.

Computer-assisted retrospective analyses of the ultrasound images were performed using custom-developed software (Quantporo; Federal University of Viçosa, MG, Brazil), specifically designed for image analysis, which calculates the brightness intensity of each pixel in a given sample area. The selected samples had a representative elementary area (REA) size of 0.25 cm^2, which means 5184 pixels were analyzed. Each pixel was equivalent to an area of 0.482 μm^2. The REA was previously determined based on a criterion proposed by VandenBygaart and Protz [34] and modified to be used for ultrasound images by Siqueira et al. [30]. Areas not representing luteal tissue (image artifacts, connective tissue, and fluid-filled cavities) were excluded from analyses.

The software generated numerical values in a scale of 256 shades of gray (0 = black and 255 = white), and these values were input into a mathematical matrix and exported to a spread-sheet program (Microsoft Office Excel 2003; Microsoft Corp., Redmond, WA, USA). The average and standard deviations of pixel brightness were assessed, and two values were considered as parameters of luteal echotexture: (1) the mean pixel value (range, 0 to 255) and (2) the standard deviation of this value, representing the pixel brightness heterogeneity within the sample area.

2.3. Blood samples

Blood samples were collected by the puncture of the coccygeal vessels, using tubes containing sodium ethylenediamine tetraacetic acid (Vacutainer Systems; Becton, Dickinson, Franklin Lakes, NJ, USA). Samples were centrifuged at 800 × *g* for 20 min at 5 °C.

After centrifugation, plasma was harvested, transferred to 1.5-mL tubes, and stored in a freezer at–20 °C until assayed. Plasma progesterone concentrations were determined with solid-phase ^{125}I radioimmunoassays (Coat-a-Count Progesterone Kit; Diagnostic Products Corporation, Los Angeles, CA, USA) and a gamma counter (Gammatec model 600; The Nucleus, Inc., Oak Ridge, TN, USA), following procedures previously validated at the Embrapa Dairy Cattle RIA Laboratory [35]. Sensitivity was 0.02 ng/mL, and the interassay and intra-assay coefficients of variation were 8.8% and 6.5%, respectively.

2.4. Embryo production systems

2.4.1. In vivo–derived embryos

Superovulations of *Bos indicus* donor cows were performed with follicle-stimulating hormone (FSH) following standard protocols described elsewhere [36,37]. Donors were artificially inseminated with commercially available semen 12 and 24 h after being detected in standing estrus. Seven days after the first artificial insemination (AI), embryos were recovered by standard nonsurgical procedures consisting of flushing both uterine horns with approximately 1 L of modified PBS (Dulbecco's phosphate-buffered saline (DPBS) + gentamicin; Nutricell, Campinas, SP, Brazil). Recovered ova/embryos were identified using a stereomicroscope (magnification ×10 to ×80) and embryos placed in

a holding medium (DPBS plus 0.2% bovine serum albumin).

2.4.2. *In vitro–produced embryos*

Cumulus-oocyte complexes (COCs) were recovered by transvaginal ultrasound-guided follicular aspiration [38,39] from live *Bos indicus* donors. Recovered COCs were maintained in culture medium (TCM 199; Invitrogen-Gibco BRL, Sao Paulo, Brazil) with HEPES during transport to the in vitro fertilization (IVF) laboratory. In vitro maturation was performed in TCM 199 with 10% inactivated estrous cow serum and 20 μg/mL FSH (Pluset, Serono, Italy) for 22 to 24 h in a humidified atmosphere of 5% CO_2 and 38.8 °C in air. Spermatozoa were obtained by swim-up [40] using Sperm TALP [41] supplemented with 6 mg/mL bovine serum albumin (BSA) fraction V. In vitro fertilization was performed in 100-μL droplets of Fert-Talp medium [41] supplemented with 20 μg heparin/mL and 6 mg/mL fatty acid–free BSA fraction V and covered with mineral oil for 18 h in humidified atmosphere of 5% CO_2 and 38.8 °C in air. The sperm concentration during fertilization was 2×10^6 spermatozoa/mL.

Sixteen hours after IVF, cumulus cells were stripped partially from oocytes by mechanical pipetting, and the putative zygotes were cultured in droplets of 50 μL CR2aa medium supplemented with 10% Fetal calf serum (FCS) and 1 mg/mL BSA and covered with mineral oil for 6 d (20 oocytes per droplet) at 38.5 °C, 5% CO_2, 5% O_2, and 90% N_2 atmosphere [42]. Half of the medium was replaced 72 h postinsemination (hpi), when cleavage rates and numbers of 8- to 16-cell embryos were determined.

2.5. *Embryo transfer*

Immediately after ultrasound examination, recipients with at least one CL and normal reproductive tract were separated from nonsuitable recipients (without a CL) and used as embryo recipients. Embryo transfer was preceded by epidural anesthesia with 3 to 5 mL lidocaine chloride 2% solution (20 mg/mL; Lidovet; Bravet Laboratory Ltda, Rio de Janeiro, Brazil). Fresh in vivo–derived (n = 90) or in vitro–produced (n = 87) embryos, in developmental stages ranging from morula to blastocyst, and quality grade 1 or 2 transferrable embryos (according to the IETS manual [43]) were transferred nonsurgically to the uterine horn ipsilateral to the ovary bearing the CL using an ET syringe with disposable sheath (IMV, L'Aigle, France) within 3 h of loading with DPBS plus 0.2% BSA in a 0.25-mL straw. During the interval between straw loading and ET, the straws were kept in a portable incubator at 37.5 °C. In vitro–produced embryos were transferred to recipients in four of the herds, whereas in vivo–derived embryos were transferred in the other four herds.

Pregnancy diagnosis was performed 23 d after embryo transfer (30 d of gestation) by ultrasonography.

2.6. *Data analysis*

Data were examined for normality with the Shapiro-Wilk test. Data that were determined to be not normally distributed were transformed into natural logarithms. Continuous outcome variables (progesterone concentrations, mean pixel value, heterogeneity, and CL area) were analyzed within each group of recipients, divided according to the type of embryo (in vivo–derived or in vitro–produced) and also with combined data from all recipients, regardless of the type of embryo. Differences among means of pregnant and nonpregnant recipients were determined using the two-sample *t*-test. The relationships between the categorical variable ''pregnancy rate'' and the continuous variables previously described were evaluated by logistic regression analysis, using the LOGISTIC procedure of the SAS software (version 9.3.1; SAS Institute Inc., Cary, NC, USA) to determine which variables were predictors of pregnancy outcome. The relationships between ''pregnancy rate'' and other categorical variables such as embryo type (in vivo or in vitro), stage of embryo development (morula or blastocyst), embryo quality (grade 1 or 2), and animal category (nonlactating cows or heifers) were analyzed by the LOGISTIC procedure as well. To assess the effect of luteal cavities on the outcome variables, combined data from all recipients were analyzed, regardless of type of embryo received, and difference among means determined using the two-sample *t*-test. The categorical variable ''pregnancy rate'' in recipients within each embryo category (in vivo–derived or in vitro–produced) was compared using chi-square analysis. Pregnancy rates among different farms and between recipients bearing cystic or noncystic corpora lutea were also compared by chi-square analysis. To verify whether high or low plasma progesterone (P_4) concentrations affected pregnancy rates, recipients within each embryo category were arbitrarily (based on what is known about physiologic concentrations of progesterone in cattle) subdivided into four categories according to their plasma P_4 concentration: (1) low (0.8 to 1.99 ng/mL); (2) fair (2.0 to 3.99 ng/mL); (3) medium (4.0 to 5.99 ng/mL); and (4) high (≥6.0 ng/mL). Pregnancy rates among these four plasma P_4

Table 1
Effect of farm and number of corpora lutea on pregnancy outcome in bovine ET recipients.

Embryo category	Pregnancy rates, % (n)				
In vitro	Farm 1	Farm 2	Farm 3	Farm 4	P value
	22.7% (5 of 22)	40.9% (9 of 22)	25.0% (6 of 24)	36.8% (7 of 19)	>0.30
In vivo	Farm 5	Farm 6	Farm 7	Farm 8	
	60.0% (21 of 35)	70.5% (12 of 17)	52.9% (9 of 17)	52.4% (11 of 21)	>0.32

	Pregnancy rates, % (n)		
	Single CL	Multiple corpora lutea	P value
In vitro	28.7% (23 of 80)	66.6% (4 of 6)	0.07
In vivo	57.9% (44 of 76)	60.0% (9 of 15)	1.0
Combined	42.9% (67 of 156)	61.9% (13 of 21)	0.11

Table 2
Mean (±SD) of different outcome variables for cystic and noncystic corpora lutea evaluated on the day of ET in bovine recipients.

End point	Number	Cystic corpora lutea	Number	Noncystic corpora lutea	P value
Mean pixel value (0 to 255)	64	71.3 ± 10.7	113	71.5 ± 12.1	0.93
Pixel heterogeneity	64	14.9 ± 3.3	113	14.4 ± 2.8	0.23
Plasma progesterone (ng/mL)	64	4.7 ± 4.0	113	4.3 ± 3.7	0.48
Luteal tissue area (cm^2)	64	3.3 ± 1.2	113	3.1 ± 1.3	0.28
Pregnancy rate (in vivo-derived)	32	56.2%	58	60.3%	0.70
Pregnancy rate (in vitro-produced)	32	43.7%[a]	55	23.6%[b]	0.056

[a,b]Within a row, means without a common superscript differed (P = 0.056).

categories were also compared using chi-square analysis. All analyses were performed using the SAS software. Statistical significance was determined based on a P value of 0.05. Data are presented as the mean ± SD of non-transformed data.

3. Results

No CL was identified in the ovaries of 62 (23.9%) of all recipients initially treated. Although at least one CL was found in the ovaries of 197 (76.1%) of the 259 recipients, only 177 cattle received embryos. The overall proportion of recipients with more than one detected CL was 10.6% (21 of 197); 85.7% (18 of 21) of those were bearing two corpora lutea, and 14.3% (3 of 21) had three. Recipients with multiple corpora lutea (two or three) were in the fair (4 of 79), medium (4 of 43), and, most of them, in the high (13 of 28) plasma P_4 category. However, 53.5% (15 of 28) of the recipients categorized in the "high P_4" group had just a single CL. The occurrence of multiple corpora lutea did not affect pregnancy rates (Table 1).

The incidence of cystic corpora lutea was 36.1%, and the presence of a central fluid-filled cavity did not affect plasma P_4 concentrations, luteal tissue area, or ultrasound attributes (mean pixel values and heterogeneity; Table 2). Within recipients receiving in vivo–derived embryos, pregnancy rates were not different (P = 0.70) in recipients that had a cystic CL from those bearing a noncystic CL. Within recipients receiving in vitro–produced embryos, however, pregnancy rate was higher in animals bearing a cystic CL than that in animals bearing a noncystic CL (P = 0.056). Comparisons of different end points among animals with cystic and noncystic corpora lutea are shown in Table 2.

The overall pregnancy rate was higher (P < 0.001) in recipients that received in vivo–derived embryos (58.8%; 53 of 90) compared with those that received in vitro–produced embryos (31.0%; 27 of 87). Results of logistic regression analysis indicated that the only variable that significantly affected pregnancy outcome was the type of embryo (P = 0.0002). No additional effects (progesterone concentration, CL echotexture, CL area, animal category, embryo quality and stage of development) met the 0.05 significance level for entry into the model. Based on the odds ratio, the likelihood of pregnancy increases in recipients transferred with in vivo–derived embryos compared with those transferred with in vitro–produced embryos (by a factor of 3.18, and a 95% confidence interval of 1.70 and 5.94; P < 0.001). Pregnancy rates did not differ among farms for either in vivo–derived or in vitro–produced embryos (Table 1; P > 0.30).

Table 3
Mean (±SD) and range of different end points retrospectively analyzed for pregnant and nonpregnant bovine embryo recipients. Examinations and sample collection were done on the day of ET.

		Pregnancy status*					
		Pregnant			Nonpregnant		
End points	Embryo category	Number	Mean ± SD	Range	Number	Mean ± SD	Range
Plasma P_4 (ng/mL)	In vivo	53	5.9 ± 5.6	0.8–27.4	37	4.4 ± 3.0	1.3–14.5
	In vitro	27	4.0 ± 2.9	1.0–14.2	60	3.5 ± 1.8	0.6–9.4
	Combined	80	5.2 ± 5.0[a]	0.8–27.4	97	3.8 ± 2.4[b]	0.6–14.5
CL area (cm^2)	In vivo	53	3.4 ± 1.5	1.1–7.2	37	3.3 ± 1.3	1.2–6.6
	In vitro	27	3.6 ± 1.5	1.8–7.8	60	3.2 ± 1.3	1.1–7.3
	Combined	80	3.5 ± 1.5	1.1–7.8	97	3.3 ± 1.3	1.1–7.3
CL echotexture: Mean pixel value (scale 0 to 255)	In vivo	53	72.4 ± 12.0	48.9–102.3	37	71.4 ± 11.3	51.7–94.8
	In vitro	27	70.6 ± 10.5	45.9–88.8	60	70.9 ± 12.0	40.0–101.6
	Combined	80	71.8 ± 11.5	45.9–102.3	97	71.2 ± 11.7	40.0–101.6
CL echotexture: Pixel heterogeneity	In vivo	53	15.1 ± 3.1	9.4–25.9	37	15.8 ± 3.0	9.9–22.2
	In vitro	27	14.3 ± 2.9	10.8–22.4	60	13.6 ± 2.7	7.6–18.6
	Combined	80	14.8 ± 3.0	9.4–25.9	97	14.4 ± 3.0	7.6–22.2

[a,b]Within a row, means without a common superscript differed ($P = 0.02$).
* Overall pregnancy rates: in vivo–derived, 58.8%; in vitro–produced, 31.0% ($P < 0.001$).

Plasma P_4 concentrations on the day of ET (8 d after the 1 mg EB treatment) were higher in animals diagnosed as pregnant compared with that in those in which pregnancy failed ($P = 0.02$). However, when recipients were divided into categories according to the type of embryo transferred (in vivo–derived, n = 90; in vitro–produced, n = 87), plasma P_4 did not differ among pregnant and nonpregnant animals ($P > 0.10$). Plasma P_4 concentrations are shown as the first end-point listed in Table 3.

Pregnancy rates in the four categories of plasma P_4 concentrations (low, fair, medium, and high) were 35.3% (6 of 17), 25% (10 of 40), 36.8% (7 of 19), and 36.3% (4 of 11), respectively, in recipients that received in vitro–produced embryos; and 70% (7 of 10), 48.7% (19 of 39), 62.5% (15 of 24), and 70.6% (12 of 17), respectively, in recipients transferred with in vivo–derived embryos. No significant difference in pregnancy rates was detected among plasma P_4 categories within each embryo category.

With respect to absolute values of CL area, there was no difference between pregnant and nonpregnant recipients receiving in vivo–derived (3.4 ± 1.5 vs. 3.3 ± 1.3 cm^2) or in vitro–produced (3.6 ± 1.5 and 3.2 ± 1.3 cm^2) embryos (Table 3; $P > 0.10$). There was also no difference in the quantitative CL echotexture (mean pixel value and pixel heterogeneity) among pregnant and nonpregnant recipients (Table 3; $P > 0.10$).

4. Discussion

In an attempt to not only enhance success rates in bovine ET but also to increase the proportion of recipients suitable to receive an embryo, protocols for FTET have recently been developed [2,4,17,44]. As estrus detection efficiency is a major concern in an ET program, the use of a FTET protocol can overcome this problem and yield satisfactory results. The proportion of suitable recipients at the time of ET previously reported, however, varied from 52.7% to 90.7% [4–6,44], which is still a broad range. The efficiency of the FTET protocol (ratio suitable/treated) observed in the current study (76.1%) was considered satisfactory, taking into account that animals underwent synchronization at unknown/random stages of the estrous cycle. Thus, it is suggested that the use of EB, exogenous progesterone, and eCG to allow self-appointed ET may be an important alternative tool for ET programs, particularly on farms with poor estrus detection efficiency.

The presence of a fluid-filled cavity is a common characteristic of the bovine CL. Several studies described the occurrence of cystic corpora lutea, with an incidence ranging from 40% to 79% [26,27,30,45]. In the current study, echotextural end points (pixel values and heterogeneity) and plasma P_4 concentrations did not differ among cystic and noncystic corpora lutea, corroborating previous reports that suggested no effect of CL cavities on functionality [45,46]. Furthermore,

fluid-filled cavities did not affect luteal echotexture (Table 2), as long as the sample for computer analysis was taken from the luteal tissue, excluding the cavity. In the current study, the area of luteal tissue (not including cavities) was similar among cystic and noncystic corpora lutea, which disagreed with a previous study [47], but agreed with another [45]. This lack of difference in the area of luteal tissue was evidence that, even when a fluid-filled cavity was present, the amount of tissue (large and small steroidogenic luteal cells) was similar. This may explain why cavities did not affect CL functionality, indirectly assessed by measurement of plasma P_4 concentrations. Although pregnancy rates were similar in recipients with cystic or solid corpora lutea that received in vivo–derived embryos, recipients bearing cystic corpora lutea had a higher pregnancy rate after receiving in vitro–produced embryos compared with those bearing noncystic corpora lutea. A previous study reported higher plasma P_4 concentration in pregnant cows bearing cystic corpora lutea compared with pregnant cows bearing homogeneous corpora lutea [47]; however, considering the fact that the presence of cavity did not affect luteal tissue area nor plasma P_4 concentration, to the best of our knowledge, there is no biological explanation for the higher pregnancy rate in recipients that received in vitro–produced embryos. Our inability to demonstrate a difference may be because of loss of statistical power due to the reduced sample size as a result of sorting recipients bearing cystic or noncystic corpora lutea.

As expected, pregnancy rate in recipients receiving in vivo–derived embryos was higher than that in recipients receiving in vitro–produced embryos (58.8% and 31.0%, respectively). These pregnancy rates after transfer of fresh embryos were in agreement with previous reports (reviewed by Holm and Callesen [48]), including data from *Bos indicus* donors [49], and demonstrated a clear effect of type of embryo (in vivo–derived vs. in vitro–produced) on fertility. Moreover, the odds of pregnancy increased by a factor of 3.18 when recipients received in vivo–derived embryos, as demonstrated by the odds ratio calculated in the current study. Also, the type of embryo was the only variable that affected pregnancy outcome, as detected by logistic regression analysis. There are several morphologic, physiologic, and metabolic differences between in vivo–derived and in vitro–produced embryos [50–52]. Also, the pattern of gene expression is likely to differ between these two types of embryos, as in vitro culture conditions are believed to influence embryonic gene expression [53–55]. Although in vitro embryo production methods have been developed very rapidly and major efforts have been directed toward the improvement of culture media and culture conditions [56,57], the transfer of fresh in vivo–derived embryos still results in higher pregnancy rates, as demonstrated in the current study. Advances in the understanding and control of oocyte maturation [58] and early embryo development (until 7 d after fertilization) would probably lead to the development of more adequate culture conditions and the production of in vitro–cultured embryos that would eventually be as viable in producing pregnancies as their in vivo counterparts.

Although combined data from all recipients enrolled in the current study showed that mean plasma P_4 concentrations were significantly higher in cattle that became pregnant than that in those who did not (Table 3), logistic regression analysis detected no effect of progesterone on pregnancy outcome. The lack of difference observed in mean plasma P_4 concentrations between pregnant and nonpregnant recipients within each embryo category (in vivo–derived or in vitro–produced) was probably a result of loss of statistical power due to the decreased sample size. The important role of progesterone in the establishment and maintenance of pregnancy is undeniable, and so a relationship between plasma P_4 concentrations and pregnancy rates would be expected (for review, see Refs. [59,60]). Interestingly, previous studies failed to show differences in progesterone concentrations on the day of ET between recipients remaining pregnant versus those that did not [18,19,23]. Moreover, the exact concentrations of progesterone that would affect (either positively or negatively) pregnancy rates are still unknown and the subject of several studies [6,24,61,62]. A previous study suggested that plasma P_4 is only related to embryo survival (higher in pregnant recipients) after Day 17 of the cycle, the time of luteolysis [32]. As blood samples were taken 7 d after arbitrary estrus in the current study, several days before the time of luteolysis, speculation regarding the usefulness of plasma P_4 measurement to predict the risk of pregnancy in embryo recipients may arise if one considers the observed difference in plasma P_4 concentrations between pregnant and nonpregnant cattle. Conversely, considering the range of values observed in this study and the lack of relationship between plasma P_4 and pregnancy detected by logistic regression, prediction of pregnancy becomes very difficult. Moreover, pregnancy was detected in recipients with plasma P_4 concentrations 7 d after estrus as low as 0.8 and as high as 27.4 ng/mL. One could speculate that this broad range of values was a result of the occurrence of multiple corpora lutea in 10.6% of the recipients enrolled in the current study; however,

similar ranges of plasma P_4 concentrations in pregnant and nonpregnant recipients were also observed in a previous study [23]. Based on this study and what was previously reported [18,19,23], plasma P_4 concentration measured on the day of ET cannot be used reliably to predict the likelihood of a successful pregnancy. Circulating progesterone concentrations, within reasonable lowest and highest limits, is not a good predictor of whether a recipient is likely to become pregnant after ET. Progesterone concentrations may be used to exclude recipients with abnormal luteal function, as suggested by Chagas e Silva et al. [32], but as long as a recipient is observed in standing estrus 6 to 8 d prior to ET and has a palpable or ultrasonically evident CL at the time of ET, it is eligible to receive an embryo. General conditions of health (disease free), nutrition (body condition score), and metabolism (postpartum interval) must also be considered as very important factors affecting pregnancy rates [1,2]. With respect to levels of plasma P_4 concentration, the subdivision of recipients based on different levels (low, fair, medium, and high) did not show significant differences in pregnancy rates among the four categories. This observation confirmed the previously reported difficulty in determining a minimum threshold of plasma P_4 required for pregnancy establishment in cattle [24] and the controversy regarding the significance of high levels of progesterone in increasing pregnancy rates [6].

Another approach to select recipients is the measurement of CL area. In the current study, however, no difference in mean CL area among pregnant and nonpregnant recipients was detected. Circulating concentrations are a result of luteal steroidogenesis (production), steroid metabolism, and clearance of progesterone [24,61]; thus the detection of a CL with a large area of luteal tissue does not necessarily mean that plasma P_4 concentration will be high. Body weight, blood volume, body fat content, and hepatic blood flow are some factors involved in steroid metabolism that influence systemic concentrations of progesterone [63,64] and may, ultimately, influence pregnancy rates. The lack of synchrony between luteal progesterone production and systemic concentrations explains the apparent controversy involving CL area (similar) and plasma P_4 (higher in pregnant) in pregnant and nonpregnant recipients enrolled in the current study. A cutoff CL diameter of 10 to 15 mm (approximately 2.5 cm^2 in area) is often used to select suitable recipients [4,6,44]; however, in the current study, 25 (31.2%) of the pregnant animals had a CL smaller than 2.5 cm^2 at the time of embryo transfer, which makes it difficult to recommend a minimum absolute CL area to be adopted as criterion for recipient suitability.

Computer-assisted analysis of ultrasound images allows quantitative assessment of CL echotexture by generating numerical values of pixel brightness intensity in a scale of 256 shades of gray [25,26,65]. The quantitative echotexture has been suggested to indicate CL functional status in both cattle [26,27,30] and small ruminants [28,29] and has potential for selection of recipients in cattle [31]. In the current study, however, there was no difference in either mean pixel brightness or pixel heterogeneity among pregnant and nonpregnant animals. To some extent, this result may just reflect the unclear relationships among CL size, progesterone concentrations, and pregnancy rates. It has been suggested that CL ultrasonographic quality is not an important end point to be used in the selection of suitable recipients, as long as a palpable CL is present and the recipient was observed in estrus 7 d before ET [23]. A previous study suggested a relationship between pixel heterogeneity of the luteal tissue and plasma P_4 concentrations [30], but this echotexture trait was significantly related to plasma P_4 only a few days before the onset of luteolysis (approximately 14 d after estrus). Taking into account this information, perhaps the day of ET (7 d after estrus) is too early to detect significant differences in echotexture between functional and nonfunctional corpora lutea. In the current study, it was not possible to define a ''desirable'' echotexture that could predict pregnancy. The potential use of CL quantitative echotextural analysis as a parameter for embryo recipient selection, consequently, was limited by the temporal sequence of endocrine interactions that provide a suitable uterine environment for embryo development and establishment of pregnancy.

In conclusion, CL size, ultrasonographic appearance, and functional traits (circulating progesterone concentrations) were poor predictors of pregnancy outcome in bovine recipients synchronized with a FTET protocol at unknown stages of the estrous cycle. The type of embryo (in vivo–derived or in vitro–produced), however, was a major factor influencing pregnancy rates. Therefore, commercial ET operators should consider this factor when deciding which type of embryo will be transferred. One must also consider that in an ET program, several factors besides the CL and plasma P_4 concentrations affect pregnancy rates.

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