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Early luteal development in Santa Inês ewes superovulated with reduced doses of porcine follicle-stimulating hormone

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Contents

The aim was to compare the early luteal development in ewes superovulated with different doses of pFSH. Twenty-nine Santa Inês ewes received a progesterone device (CIDR®) for 8 days. Gonadotrophic treatment started on Day 6: G200 (control, n = 9, 200 mg); G133 (n = 10, 133 mg); and G100 (n = 10, 100 mg of pFSH). On Day 6, all females received eCG (300 IU). B-mode and spectral Doppler ultrasonography were performed daily during the early luteal phase (Days 11–15) to monitor the development of corpora lutea (CLs; dimensions) and ovarian arteries indices. CLs were also classified as normal or prematurely regressed (PRCL) on Day 15 by videolaparoscopy. Ewes from G100 and G133 showed gradual increase in luteal diameter during the early luteal phase ($p < 0.001$), whereas G200 animals presented increase from Day 11 to Day 13, and then decrease on Days 14 and 15 ($p < 0.001$). The G200 females showed greater percentage of PRCL (45.20%) than those of the other groups ($p < 0.001$). The normal CLs number was greater in G100 than in G133 ($p = 0.04$), while the PRCL number was greater in G200 than in the other groups ($p = 0.03$). Resistive index (RI) was greater in G200 than in G100 ($p = 0.02$). RI was lower in Day 12 than Day 15 ($p = 0.02$). Pulsatility index (PI) was greater on Days 14 and 15 ($p < 0.01$). In conclusion, the lowest dose of pFSH (100 mg) can be considered sufficient for an efficient superovulatory response in sheep, producing better CLs development dynamic in early luteal phase and ovarian blood perfusion and smaller number of PRCL than the traditional (200 mg) pFSH dose.

KEYWORDS

corpus luteum, ovarian artery, ovine, premature luteolysis, spectral doppler indices

1 | INTRODUCTION

In vivo embryo production is a powerful technology to accelerate genetic improvement and increase reproductive efficiency in small ruminants (Amiridis & Cseh, 2012). To produce embryos in vivo, Multiple Ovulation and Embryo Transfer (MOET) programs are used, which present limitations on cost, methodology, incidence of premature regression of corpora lutea (PRCL) and heterogeneous response to exogenous follicle-stimulating hormone (FSH), then resulting in variable and/or reduced number of viable embryos produced (Menchaca et al., 2010).

In small ruminants superovulatory protocols, the total doses of pFSH (Folltropin®, Bioniche, Canada) more frequently used are 256 mg (Oliveira et al., 2012), 215 mg (Sánchez-Dávila et al., 2014) or 200 mg (Loiola Filho et al., 2015; Oliveira et al., 2014) while in cows good results are achieved with lower doses (100–133 mg) (Baruselli et al., 2006). Therefore, studies addressing the efficacy of lower doses of FSH in sheep are needed. Because, high doses of FSH may induce changes in ovarian and/or endocrine dynamics (Kafi & McGowan, 1997) and on LH pulses, increasing the incidence of PRCL (Bevers, Dieleman, Blankestein, Tol, & Broek, 1989; Oliveira et al., 2009) besides being more expensive (Loiola Filho et al., 2015). Furthermore, higher doses may maintain the stimulus of the luteinized anovulatory follicles for longer periods, so these follicles are still producing oestrogen in the early luteal phase, causing persistence of high concentrations of this hormone, providing the early release of PGF2α and thus premature luteal regression (Okada et al., 2000).

When the physiological pulsatile secretion of PGF2α is released earlier than the normal period in the oestrous cycle, corpora lutea (CL) regress before embryo collection, which can occur in approximately 75% of the treated ewes (Saharrea et al., 1998). Although the mechanisms determining the PRCL are not yet fully understood, the gonadotrophin used, commercial product, manufacturing batch, FSH:LH ratio of the preparation, protocol of injection and/or use of high exogenous FSH doses may influence on this incidence (Kafi & McGowan, 1997; Quan et al., 2011). There are few reports in the literature regarding studies with lower doses of FSH in sheep (Loiola Filho et al., 2015; Sánchez-Dávila et al., 2014; Wu et al., 2011), moreover there is no disclosure of results concerning the effects on luteal development dynamics, as well as about the occurrence of PRCL. We hypothesized that the incidence of PRCL decreases when lower doses of exogenous pFSH are used in embryo donor sheep. Thus, this study was designed to compare the luteal ovarian dynamics, blood flow indices of ovarian arteries and occurrence of PRCL after three different pFSH doses in superovulated ewes.

2 | MATERIALS AND METHODS

2.1 | Location, animals and experimental procedures

Procedures were approved by the ethics commission of the São Paulo State University Jaboticabal, São Paulo, Brazil under protocol nº 12062/14.

Twenty-nine adult multiparous mean of 3 ± 1 years old and 45.17 ± 5.76 kg of body weight (BW) and 2.5–3.5 of body condition score (range 1–5 with 0.25 steps), healthy (normal physical, obstetrical and haematological examination, without history of reproductive diseases) Santa Inês ewes were used. These animals were kept in a sheepfold, fed with corn silage, balanced commercial concentrate, mineral salt and water ad libitum.

The animals were submitted to superovulatory protocols, initiated on a random day of the oestrous cycle or the anovulatory period (Day 0). On this day, an i.m. injection of 2.5 μg/kg of PGF2α analogue (Cloprostenol; Sincrocio®, Ouro Fino, Brazil) was administrated and an intravaginal device containing 0.3 g of progesterone was inserted (Eazi-Breed CIDR®; Pfizer Inc., Auckland, New Zealand), which remained until Day 8. On Day 6 (48 hr prior to CIDR® withdrawal), gonadotrophic treatment was initiated. At this moment, the ewes were randomly divided into three experimental groups according to the total dose of exogenous porcine FSH (pFSH; Folltropin®): G200 (control, $n = 9$, BW: 45.0 ± 5.9 kg)—200 mg; G133 ($n = 10$; BW: 45.8 ± 5.7 kg)—133 mg and G100 ($n = 10$; BW: 45.3 ± 5.8 kg)—100 mg. These doses were administered in eight i.m. injections at 12 hr intervals (20%, 20%, 15%, 15%, 10%, 10%, 5% and 5% of the pFSH). On Day 6 (1st pFSH injection), females also received 300 IU of eCG (Novormon®; Coopers, MSD, Brazil). On Day 8, another equal dose of the PGFα analogue was i.m. injected assuring luteolysis of the remaining corpora lutea (Fonseca et al, 2008). The doses tested in the present study were defined according to better usage of the drug based on its presentation (400 mg of pFSH in 20 ml). Thus, in G200, G133 and G100 it was possible to use one vial for two, three and four females, respectively.

2.2 | Hormone assays

Jugular blood samples were collected immediately before each ultrasonographic examination, from Day 11 to 15 (early luteal phase), to determine serum concentrations of progesterone (P4). Blood samples (10 ml) were drawn into evacuated blood collection tubes without anti-coagulants (Becton Dickinson Diagnostics, São Paulo, Brazil). All samples were then centrifuged at 3,000 *g* for 15 min, and sera were separated into aliquots properly marked and stored at −20°C until assay at a later date. Progesterone concentrations were measured using a commercial kit (MP Biomedicals, LLC, Diagnostics Division, Orangeburg, NY, USA), according to the manufacturer's specifications. The assay sensitivity was 0.1 ng/ml, and the range of standards was from 0.1 to 80 ng/ml. All serum samples were analyzed in a single assay with the 18% coefficient of variation. All data were within maximum and minimum points of the curve.

2.3 | Ultrasonographic techniques and videolaparoscopy

B-mode ultrasound evaluations of the CLs formed after the superovulatory protocols and spectral Doppler evaluations of the ovarian arteries were performed in all animals in the mornings starting 07:00, maintaining the same animal sequence, at 24 hr intervals during the early luteal phase from Day 11 (i.e., equivalent to the period of luteogenesis; Day 1 after ovulation) to Day 15 (i.e., equivalent to the day of embryo collection; Day 5 after ovulation). Ovulations occurred on average 42.2 ± 11.9 hr after CIDRs® removal (i.e., between Days 9 and 10 of the protocols). The Colour Doppler modality was used to aid in the identification of CLs.

The MyLab Vet30 Gold (Esaote, Italy) ultrasound equipment was used with a multifrequential linear transrectal transducer (7.5 MHz), coupled to an extender to allow its manipulation and equipment configurations were standardized. Animals were

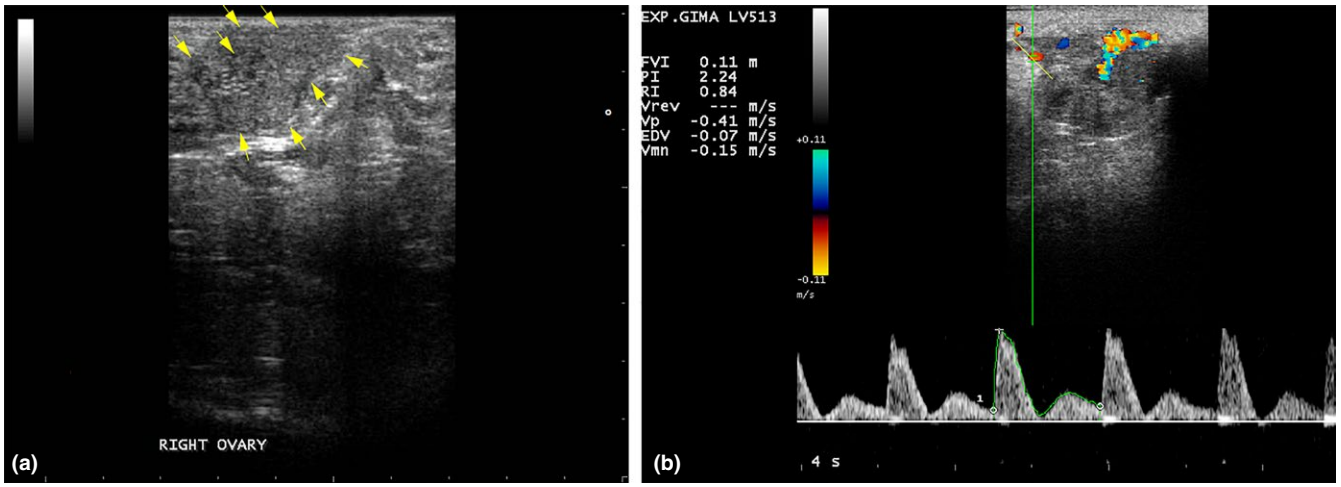

FIGURE 1 (a) Ovarian B-mode ultrasonography on Day 15 (five to six days after ovulation) of the superovulatory protocol in Santa Inês ewe, with the corpora lutea indicated by the yellow arrows. (b) Spectral Doppler ultrasonography of the ovarian artery to obtain blood flow indices (PSV, EDV, RI and PI) of superovulated ewe. EDV, end diastolic velocity; PI, pulsatility index; PSV, peak systolic velocity; RI, resistance index

restrained in a quadrupedal position with the abdominal wall compressed by the examiner's leg. Faeces were removed from the rectum, and acoustic gel was used. After transrectal introduction, uterus and ovaries were located (Oliveira et al., 2014). In B-mode examinations, the main gain was set to 65% of a maximum value and a single focal point was positioned in the region of interest; all settings including the near and far gain and contrast were kept constant throughout the study period. Doppler sampling frequency (PRF) of 1.4 kHz, colour gain equal to 70% of maximum value or just below the background noise level recorded in a standing motionless animal, wall filter (WF) of 75 KHz and depth of 8 cm, these settings were standardized and maintained in all examinations. Videos of the B-mode and colour Doppler ovarian scans were recorded for the identification of the luteal structure.

The detected CLs were quantified, and their mean of the maximum diameters measured (Figure 1a), considering the equation: $D = (A + B)/2$; *A*—represents the maximum vertical dimension and *B*—the maximum horizontal dimension. Luteal structures were identified according to their position in the ovary to allow daily monitoring. Cineloop function was used to select frames with the largest diameter of each corpus luteum.

Spectral Doppler ultrasonography was performed to evaluate blood flow indices of the ovarian arteries (Figure 1b). After identification of the ovarian artery by colour Doppler ultrasound, a sample volume (gate) had its size adjusted (2–4 mm) was positioned in the central portion of the artery. A minimum of three consecutive waves/artery were used in the evaluation. According to previous studies (Ginther & Utt, 2004), the angle of insonance used was between 30 and 60°. Then, with the mapping free of artefacts, images were frozen and waveform analysis was performed to determine the systolic peak velocity (SPV), end diastolic velocity (EDV), the resistance index (RI = PSV−EDV]/SPV) and the pulsatility index (PI = [SPV−EDV]/mean velocity).

On the day of embryo collection (5 or 6 days after ovulation—Day 15), videolaparoscopy procedures were performed according to Oliveira et al. (2012) to evaluate ovarian structures (Figure 2), luteinized anovulatory follicles (luteal structures ≥5 mm and lacking ovulatory stigmata; Bartlewski et al., 2017) and CLs. All visualized structures were quantified. CLs were also macroscopically classified, according to morphological characteristics indicative of their functionality, as normal or prematurely regressed CLs (PRCL, corpora lutea with a pale appearance, smaller in size than normal CLs [<6 mm]

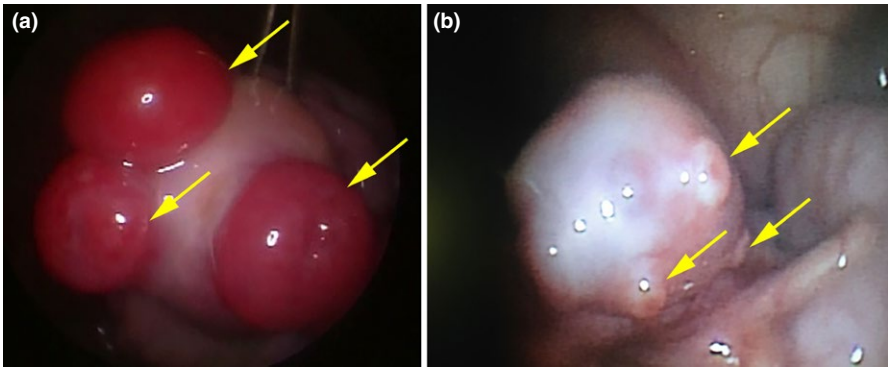

FIGURE 2 Corpora lutea observed during videolaparoscopy on Day 15 (5–6 days after ovulation) in Santa Inês ewes superovulated with different doses of pFSH. Corpora lutea are indicated by the yellow arrows. (a) Normal corpora lutea. (b) Prematurely regressed corpora lutea

TABLE 1 Means and standard deviations of the diameters (mm) of corpora lutea during early luteal phase in ewes subjected to superovulation with 100 (G100), 133 (G133) or 200 (G200) mg of exogenous pFSH

	G100	G133	G200	*p* value
Day 11	5.21 ± 1.10^{Aa}	5.72 ± 1.03^{Aa}	5.23 ± 0.86^{Aa}	0.93
Day 12	6.10 ± 1.40^{Bab}	6.41 ± 1.17^{Ba}	5.67 ± 1.04^{ABb}	0.001
Day 13	6.78 ± 1.80^{Ca}	6.69 ± 1.51^{Ca}	6.43 ± 1.32^{Ca}	0.90
Day 14	6.80 ± 1.80^{CDa}	6.95 ± 2.02^{CDa}	5.75 ± 1.57^{Bb}	<0.001
Day 15	6.99 ± 2.02^{Da}	7.23 ± 2.06^{Da}	5.86 ± 1.79^{Bb}	<0.001
p value	<0.001	<0.001	<0.001	

Note. Lowercase letters indicate differences between experimental groups (total dose of pFSH), and uppercase letters indicate differences between evaluation days. D = day, being D0 = insertion of the CIDR® device, beginning of superovulatory protocols, Day 11 = 1 or 2 days after ovulation, and Day 15 = corresponding day of the embryo collection.

and not protrude) (Rubianes et al., 1996) and the percentage of PRCL was calculated dividing the PRCL number by the total CLs times 100.

2.4 | Statistical analysis

The R® software (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. Experimental design constituted completely randomized design with subdivided parcel times (moments). Residual normality and homoscedasticity of variances were previously tested (Shapiro–Wilk and Bartlett tests). CLs diameter, serum progesterone concentration and ovarian arteries blood flow indices were compared between treatments (pFSH doses), moments (days) and their interactions by repeated ANOVA, if results were significant, means were compared by Tuckey's test. The number of ovarian structure variables, where there was not a normal distribution, was compared between treatments by Kruskal–Wallis test and Dunns post-test. The CLs diameter and P4 concentration were correlated using the Pearson correlation test. And the rates of regressed and non-regressed CLs were compared between treatments using the Fisher's exact test. The level of significance was set at $p < 0.05$ for all tests.

3 | RESULTS

In all ewes, it was possible to visualize the CLs by ultrasound evaluations from the first day after ovulation (Day 11).

Females treated with 100 and 133 mg of pFSH showed a gradual increase ($p < 0.001$) in the diameter of the CLs throughout the days after ovulation, whereas the animals of G200 presented an early increase in luteal diameter from Day 11 to Day 13, followed by a decrease ($p < 0.001$) on Day 14 and Day 15 and the diameter of these CLs being lower ($p < 0.001$) in G200 on Days 14 and 15 (Table 1).

Positive correlation was observed between the mean diameter of the CLs and serum P4 concentration ($r = 0.650$, $p < 0.001$). However, on the last evaluation day (Day 15, corresponding day of the embryo collection) the serum P4 concentration was higher ($p = 0.0481$) in G100 females than those of the other groups (Figure 3). The serum P4 concentration was higher ($p = 0.0158$) in animals without than with occurrence of PRCL (11.99 ± 9.98 vs. 4.06 ± 3.67, respectively).

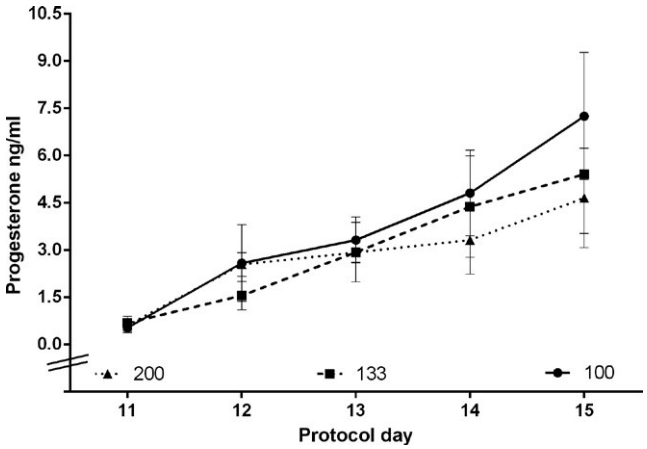

FIGURE 3 Serum progesterone concentration (ng/ml) in the early luteal phase of ewes receiving 100, 133 or 200 mg of exogenous pFSH in the superovulatory treatment. Day 11 = the first or second day after ovulation; Day 15 = day of the embryo collection. *indicates significance in G100 values in relation to the other groups ($p < 0.05$, Tukey's test)

Regarding the evaluation of vascular indices of the ovarian arteries, the RI was higher ($p = 0.02$) on Day 15 than on Day 12 and was higher ($p = 0.02$) in G200 than in G100. The PI was higher ($p < 0.01$) on Days 14 and 15 than on the other days and similar ($p > 0.05$) between groups. In addition, PSV and EDV were similar ($p > 0.30$) between treatments and moments (Table 2).

The ovarian responses of superovulated ewes are presented in Table 3. The total CLs were higher ($p = 0.04$) in G200 than in G133. Normal CLs were higher ($p = 0.04$) in the G100 animals than in the G133. The PRCLs were higher ($p = 0.03$) in G200 than in another groups. The luteinized anovulatory follicles were similar ($p = 0.85$) among treatments.

The percentage of CLs that regress prematurely (PRCLs * 100/ Total CLs) was higher ($p < 0.001$) in G200 (45.20%) than in G133 and G100 (30.11% and 19.84%, respectively).

4 | DISCUSSION

The gradual increase of CLs diameter during the early luteal phase observed in animals from G100 and G133 indicates adequate

TABLE 2 Mean and standard deviations of blood flow indices of ovarian arteries during early luteal phase in ewes subjected to superovulation with 100 (G100), 133 (G133) or 200 (G200) mg of exogenous pFSH

	PSV	RI	PI	EDV
Day 11	0.07 ± 0.26	0.74 ± 0.12^{A}	1.88 ± 0.85^{A}	0.03 ± 0.06
Day 12	0.00 ± 0.30	0.72 ± 0.11^{A}	1.63 ± 0.56^{A}	0.01 ± 0.08
Day 13	0.03 ± 0.31	0.75 ± 0.09^{AB}	1.83 ± 0.53^{A}	0.02 ± 0.08
Day 14	0.08 ± 0.29	0.77 ± 0.09^{AB}	2.08 ± 0.69^{B}	0.03 ± 0.08
Day 15	0.08 ± 0.30	0.81 ± 0.29^{B}	2.06 ± 0.74^{B}	0.02 ± 0.07
P value	0.50	0.02	<0.01	0.43
G100	0.04 ± 0.29	0.74 ± 0.12^{a}	1.77 ± 0.70	0.03 ± 0.08
G133	0.04 ± 0.28	0.75 ± 0.10^{ab}	1.88 ± 0.68	0.02 ± 0.08
G200	0.08 ± 0.32	0.81 ± 0.23^{b}	2.04 ± 0.69	0.01 ± 0.06
p value	0.56	0.02	0.09	0.30

Notes. PI: pulsatility index; PSV: peak systolic velocity; RI: resistance index.
Lowercase letters indicate differences between experimental groups (total dose of pFSH), and uppercase letters indicate differences between evaluation days. D = day, being Day 0 = insertion of the CIDR® device, beginning of superovulatory protocols, Day 11 = 1 or 2 days after ovulation, and Day 15 = corresponding day of the embryo collection.

TABLE 3 Mean and standard deviations of luteinized anovulatory follicles (LUFs), total corpora lutea (CLs), normal CLs and prematurely regressed corpora lutea (PRCL) observed by videolaparoscopy examination on the corresponding day of embryo collection in superovulated ewes with 100 (G100), 133 (G133) or 200 (G200) mg of exogenous pFSH

	G100	G133	G200	*p* value
LUFs	3.00 ± 3.97 (min 1, max 11)	2.30 ± 1.70 (min 1, max 5)	3.22 ± 5.63 (min 0, max 18)	0.85
Total CLs	12.60 ± 5.72^{ab} (min 7, max 24)	9.30 ± 3.71^{b} (min 4, max 15)	15.00 ± 6.50^{a} (min 5, max 26)	0.04
Normal CLs	10.10 ± 7.52^{a} (min 0, max 24)	6.50 ± 4.77^{b} (min 0, max 15)	8.22 ± 8.60^{ab} (min 0, max 26)	0.04
PRCL	2.50 ± 4.38^{b} (min 0, max 12)	2.80 ± 4.78^{b} (min 0, max 14)	6.78 ± 8.09^{a} (min 0, max 22)	0.03

Note. Letters indicate difference between experimental groups (total dose of pFSH) ($p < 0.05$).

development of these structures based on the information brought by some authors (Gonzalez de Bulnes et al., 2000; Kraisoon et al., 2015), who described progressive increasing of luteal area and CL weight, respectively, until mid-luteal phase in sheep. Conversely, animals which received 200 mg of pFSH presented an increase in the diameter of CLs only between Days 11–13 and then, reduction on Days 14 and 15. It is suggested that the change in the growth of CLs is related to the loss of their functionality (P4 <1 ng/ml), due to the higher rate of PRCLs in the G200 group, the correlation between CLs size and P4 concentration, and the higher serum P4 concentration in ewes without than with PRCL occurrence. According to Balaro, Santos, Moura, Fonseca, and Brandão (2017), luteal biometry presents a gradual and progressive decrease in the luteal regression phase, whereas the luteal blood flow decreases abruptly in association with P4. These authors indicated that the luteal blood flow values were more reliable in predicting the CL functionality when compared to the luteal biometry data. Differentiating functional CLs from PRCL is not yet described using only ultrasound evaluation methods (Oliveira et al., 2018), but nonfunctional CLs appear to have smaller diameters and other morphological characteristics at laparoscopic evaluation (Rubianes et al., 1996).

It is suggested that the use of higher doses of FSH may lead to the persistence of luteinized anovulatory follicles, which continue to produce oestrogen during the early luteal phase and promote the early release of PGF2α and luteal regression (Okada et al., 2000). However, in the present study no greater number of anovulatory follicles were observed in the G200 in relation to the other groups. It was not intended in our study to determine the oestrogen concentrations that these follicles would be producing; therefore, it is not possible to indicate the differences in steroidogenic activity of the luteinized anovulatory follicles present among ewes of the different groups, nor whether it was responsible for the variation in the percentage of premature regression of CLs between groups. We emphasize the need for further research on the connection between the steroidogenic capacity of the luteinized anovulatory follicles and the occurrence of premature luteolysis. Furthermore, the number of luteinized anovulatory follicles does not appear to be related to the dose of pFSH used in the superovulatory protocol and other factors must be involved. Despite this, it is known that its presence affects the superovulatory response and is one of the factors related to the high variability in viable embryo production in sheep (Veiga-Lopez et al., 2006). Oliveira et al. (2012) observed that supplementation with LH at the end of the gonadotrophic treatment increases the

frequency of CLs and decreases the percentage of luteinized anovulatory follicles; however, the authors suggest that the effect of changing the FSH/LH ratio in superovulatory protocols at ovulation rate is controversial and seems to vary according to different breeds.

The ovarian superstimulation with pFSH dose might contribute to the variability in superovulatory responses in ewes (Bartlewski, Fuerst, Alexander, & King, 2009). The isolated effect of pFSH dose reduction was not able to reduce or eliminate the large variation of superovulatory responses between animals. Despite this, we observed that superovulated ewes with 100 mg of pFSH demonstrated more adequate development of luteal structures when compared to those treated with 200 mg, as can be seen by the lower number and percentage of PRCL, better dynamic of luteal development (i.e., gradual and progressive increase of diameters) and higher concentration of progesterone on Day 15. From these findings, it can be expected that the production of embryos can be favoured when the lowest dose of pFSH is used. Although more studies are needed to confirm our hypothesis, the number of ovulations is decisive for the embryo yields as well as the luteal dynamic, especially the adequate progesterone concentrations until embryo collection day are beneficial to the uterine environment and embryonic development in donor females (Connell et al., 2013; Kraisoon et al., 2015). Brasil et al. (2016) observed a similar number of CLs to that obtained in this study using 133 mg of total dose of pFSH in Santa Inês ewes. Sanchez-Dávila et al. (2014) reported similar number of CLs in response to 145 and 215 mg of total dose of pFSH for superovulation in goats whereas lower ovarian response was recorded when 80 mg was used; the same was recorded to produce transferable embryos. The number of CLs observed in this experiment was greater than observed by Oliveira et al. (2017) with the same protocol of superovulation in Santa Inês ewes using 200 mg of total dose of pFSH. Therefore, the reduction of total dose of pFSH in relation to the commonly used in small ruminants (200–256 mg; Oliveira, 2011) points to be beneficial; however, there seems to be a limit to this reduction.

The blood flow indices of the ovarian arteries reflect the haemodynamics of the ovarian tissue (Ginther & Utt 2004). Physiologically, during luteolysis, the release of PGF2α pulses leads to an acute increase in blood flow, followed by a decrease due to local release of vasoactive peptides and consequent vasoconstriction (Acosta and Miyamoto, 2004). The resistance index correlates negatively with ovarian perfusion (Ginther & Utt 2004; Ginther et al, 2013) and in bitches, a decrease in this index was observed at the early luteal phase (Köster et al., 2001). Thus, the higher value of RI in G200 animals than in G100 seems to be related to higher occurrence of premature luteolysis observed among females receiving the highest pFSH dose. This fact is substantiated by the observation of progressive decrease in ovarian blood supply during luteolysis in heifers (Ginther et al, 2013). Additionally, a study reports that abnormal ovarian hemodynamic pattern may be a result of altered pulsatility of LH, dependent on the prostaglandin release (Parsanezhad et al., 2003). This indicates that the Doppler fluxometric indices can be correlated with the hormonal changes that occur during these processes. It is known that LH support is essential for CL formation, primarily for initial growth and cell differentiation (Bartlewski, Baby, & Giffin, 2011).

On the other hand, the greater blood supply inferred by the lower vascular resistance in the G100 can be related to a more effective luteogenesis process, since the main function of the angiogenic factors produced in the CLs is to guarantee the functionality and growth of this structure (Reynolds et al., 2000), and it is probably a plausible explanation for the larger size of CLs observed in this group when compared to the G200. The decrease in RI and PI reflects an increase in the vascular perfusion of the ovarian tissue (Ginther & Utt 2004), demonstrating that the lower value in RI on Day 12 in relation to Day 15 and in PI on Days 11, 12 and 13 observed in the present study (representing 2–3 days after ovulation) can be explained by the intensity of the angiogenic process of CL reaching the peak of 2–3 days after ovulation (Reynolds et al., 2000). Our results are in accordance with the decrease in RI and PI at 2 days after ovulation observed in the early luteal phase in bitches (Köster et al., 2001). In addition, FSH administration may induce the expression of the vascular endothelial growth factor (VEGF), the main mitogenic factor of endothelial cells produced by the corpus luteum, which induces cell migration, differentiation and proliferation, as well as maturation and stabilization of blood vessels (Zhang et al., 2013), promoting a better quality of luteogenesis related to lower RI values on Day 12 compared to Day 15. The progressive increase in luteal vascularization and progesterone concentration observed in this study corroborates the study by Figueira et al. (2015) which demonstrated the progressive growth of luteal vascularization accompanied by progesterone until the fourth day after ovulation in Santa Inês ewes, as well as the maximum growth of angiogenesis between days 2 and 3 in cattle (Reynolds et al., 2000).

The PI showed to be the first marker for the diagnosis of premature regression in heifers, since females presenting CLs of this category showed higher values of this index in 4th and 6th days after induction of ovulation (Vrisman et al., 2018). In the present study, PI values were higher on Days 14 and 15 than on Days 11, 12 and 13, which may also be related to regression of part or all of CLs in most (41.35%) of our females. However, no difference was observed between the experimental groups, suggesting that the variation of indices apparently is not determined by the percentage of regressed CLs. In superovulated cows, a progressive decline in ovarian artery PI was observed during the luteal phase (Honnens et al., 2009), in opposition to what was observed in the present study. The difference may be explained by the fact that superovulation in cows is not related to the occurrence of CL regression as in the sheep.

5 | CONCLUSION

The present results indicate that superovulatory treatment with a total dose of 100 mg of pFSH in ewes results in better CLs development dynamic in early luteal phase and ovarian blood perfusion when compared with 200 mg of pFSH. In addition, although the lowest dose of pFSH does not prevent the high variability in superovulatory responses, it reduces the occurrence of prematurely regressed corpora lutea in relation

to traditional pFSH dose. The total 100 mg dose of pFSH is recommended in ovine superovulation because it results in better cost/benefit.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

The designed study was performed by Mariana Garcia Kako Rodriguez, Maria Emília Franco Oliveira and Jeferson Ferreira Fonseca. In the experimental period, the samples were collected by Mariana Garcia Kako Rodriguez, Giovanna Serpa Maciel, Victor José Correia Santos, Priscila Del Aguila da Silva, Ricardo Andrés Ramirez Uscategui, Ricardo Perecin Nociti and Marcus Antonio Rossi Feliciano. The results obtained were analyzed by Mariana Garcia Kako Rodriguez, Ricardo Andrés Ramirez Uscategui and Felipe Zandonadi Brandão. The preparation of the manuscript and its correction was carried out by all authors.

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