Reproduction in Domestic Animals

Reprod Dom Anim **50**, 643–650 (2015); doi: 10.1111/rda.12543
ISSN 0936–6768

Colour Doppler Ultrasonography as a Tool to Assess Luteal Function in Santa Inês Ewes

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Contents
The aim of this study was to evaluate luteal dynamics in the Santa Inês ewes using colour Doppler (CD) ultrasonography. Oestrus was synchronized in nulliparous females (n = 18), and subsequently, they were only teased (n = 6) or teased and mated (n = 12). Blood samples were collected daily for plasma progesterone (P_4) concentrations. Ultrasonographic images of corpora lutea (CL) in CD mode were obtained for further analysis in its largest diameter. The CD mode allowed an early sequential monitoring of CL that was visualized by the first time 0.77 ± 0.62 days after ovulation, with luteal area 29.68 ± 13.21 mm^2. During the luteogenesis, a progressive increase was observed, followed by a plateau of luteal area, vascularization area and plasma concentrations of P_4 reaching maximum values in D11 (124.0 ± 38.0 mm^2, 52.78 ± 24.08 mm^2 and 11.23 ± 4.89 ng/ml, respectively). In the luteolysis, the plasma concentrations of P_4 decreased sharply, whereas luteal and vascularization area gradually. The vascularization area was positively correlated with plasma concentrations of P_4 during the luteogenesis ($r = 0.22$) and luteolysis ($r = 0.48$). The luteal dynamics of Santa Inês ewes showed patterns similar to those observed in other sheep breeds studied. The CD ultrasonography has the potential to be used as a tool to assess luteal function in sheep.

Introduction

The corpus luteum (CL) is a transitory gland whose development in the ovary begins immediately after ovulation. Its main function is to synthesize progesterone (P_4), a steroid hormone essential for establishment and maintenance of pregnancy in several domestic species (Niswender et al. 2000). In sheep, studies with ovariectomized animals demonstrated that P_4 is important for pregnancy since the first days after mating (Miller and Moore 1976; Wilmut et al. 1985). Therefore, the presence of a functional CL in embryo donors and recipients is a basic requirement in multiple ovulation and embryo transfer (MOET) programmes (Bari et al. 2003), among other applications in animal assisted reproduction.

The association between B-mode ultrasonography and radioimmunoassay markedly increased the knowledge about the biology of reproduction, demonstrating that a morphological change is directly related with hormonal and other functional variations (Griffin and Ginther 1992). Previous studies in ewes observed a positive correlation between luteal morphological parameters (CL area and diameter) and plasma progesterone concentration (Bartlewski et al. 1999; Gonzalez-Bulnes et al. 2000). However, luteal function assessment by morphological measurement using B-mode ultrasonography has limitations (Pieterse et al. 1990), specially during luteolysis period (Kastelic et al. 1990; Bartlewski et al. 1999). Computer-assisted image analysis was previously tested as a new methodology to assess luteal function in domestics ruminants (Davies et al. 2006; Siqueira et al. 2009; Arashiro et al. 2010a); yet, it did not provide a strong indicator, demonstrating a limited application of this technology.

New technologies in diagnostic image tools, such as Doppler ultrasonography, have been shown to be promising (Singh et al. 2003). As vascularization is directly associated with organ functionality, the association of Doppler technology with B-mode ultrasonography can provide not only morphological data but also information regarding the functional status of the organ, including those from reproductive tract (Miyamoto et al. 2006). In ruminants, Doppler ultrasonography has been used mainly in cattle to evaluate the blood flow in the uterine artery during oestrous cycle (Bollwein et al. 2000) and pregnancy (Bollwein et al. 2002; Honnens et al. 2008), to predict fertility (Siddiqui et al. 2009), and in early pregnancy diagnosis (Utt et al. 2009; Siqueira et al. 2013). In sheep, the Doppler was used to assess small vessels of the ovary (Marret et al. 2006), vascular umbilical cord (Panarace et al. 2008; Domínguez et al. 2013), ovulatory follicles (El-Sherry et al. 2013) and superovulatory response (Oliveira et al. 2014).

During luteogenesis and luteolysis periods, the luteal tissue remodelling observed includes the formation and regression of blood vessels. Previous studies in cattle showed a close association between the vascularization of CL and the potential for P_4 production (Acosta et al. 2003), as the vascular system provides oxygen, nutrients, hormones and substrates necessary for steroidogenesis. Colour Doppler ultrasonography was previously used in cattle to assess vascular parameters of the CL during its development and regression (Acosta et al. 2002, 2003; Ginther et al. 2007). To our knowledge, colour Doppler ultrasonography has not been studied yet as a tool to evaluate luteal function in sheep. Thus, the present study aimed to assess the luteal dynamics and evaluate the use of colour Doppler ultrasonography as a tool to assess luteal function in Santa Inês ewes.

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Materials and Methods

Experimental conditions and animals

This study was approved by the Ethics Committee for Research Involving Animals – Universidade Federal Fluminense (UFF), protocol no 191. The experiment was conducted in the Farm School of UFF, located at Cachoeiras de Macacu, Rio de Janeiro State, Brazil (22°31′16″ S and 42°42′31″ W). Eighteen nulliparous ewes (*Ovis aries*) of Santa Inês breed with mean body condition score (BCS; Menzies 2007) and body weight, respectively, of 3.1 ± 0.4 and 46.9 ± 6.4 kg were used. The reproductive history of the animals used included normal cyclic ovarian luteal activity and no diseases of the reproductive tract detectable by transrectal ultrasonography and vaginoscopy.

The animals were kept in a semiconfined system with access to rotational grazing in Tifton 85 (*Cynodon dactylon*) and fed with chopped Napier grass (*Penisse-tum purpureum v. Cameron*) and balanced concentrate supplement during the experiment to meet the requirements for maintenance (NRC, 2007). Water and mineralized salt (Presencefós©, Presence Animal Nutrition, São Paulo, Brazil) were available *ad libitum*.

Synchronization of oestrus and mating

Oestrous cycle was synchronized using 60 mg medroxyprogesterone acetate sponge (Progespon® – MSD Animal Health, São Paulo, Brazil) for 6 days. At 24 h before sponge removal, 37.5 μg d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil) and 300 IU eCG (Novormon 5000®, MSD Animal Health, São Paulo, Brazil) were given.

After the synchronization protocol, oestrous detection was performed twice a day (7:00 am and 7:00 pm) aided by a teaser male (lateral penile deviation). Twelve females were mated with two Santa Inês rams of proven fertility, whereas six ewes were only teased. Females were considered to be in oestrus when allowed to be mounted. Pregnancy was diagnosed 30 days after natural mating by ultrasonography.

B-Mode and Colour Doppler Ovarian ultrasonography

Transrectal ovarian ultrasonography was performed for the entire experiment by the same operator. After oestrous onset, B-mode ultrasound examinations using a portable equipment (Sonoscape S6, Sonoscape©, Shenzhen, China) coupled to a 7.5 MHz linear array transducer was performed. To facilitate manipulation of the transducer, it was taped to a PVC tube. Does were maintained in a standing position, faecal pellets were removed manually (with a finger), and 20 ml of carboxymethylcellulose gel (Carbogel®, São Paulo, Brazil) was placed into the rectum with a syringe. The examinations were performed once a day (between 8:00 am and 10:00 am) to evaluate the pre-ovulatory follicle and detect the moment of ovulation (D0). The moment of ovulation was characterized as the day when the largest ovarian antral follicle(s) visualized on the previous day was (were) no longer observed.

After ovulation, luteal dynamics was evaluated with colour Doppler ultrasonography using the same equipment and examination scheme previously described. The localization of the ovaries was performed using B-mode. After that, dual screen mode (B-mode and colour Doppler) was activated and all CL present in both ovaries were individually evaluated with slow and continuous scan. The assessment of each CL was video-recorded (.Cin format). Ultrasonographic evaluation of the CL was performed until the visualization of an embryonic vesicle (approximately 20 days) or the occurrence of a new ovulation (approximately 18 days). The Doppler settings used in the luteal assessments were the same throughout the experiment, as follows: 75% colour gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth and wall filter (WF) of 75 MHz. An schematic representation of the experimental design is demonstrated in Fig. 1.

Image analysis

After the sonograms, using the ultrasound equipment, each video was reviewed and cross-sectional images of the CL were obtained in its largest diameter to measure the total luteal area and vascularization area.

Image analysis was performed later in a dark place. The areas were measured (mm²) using the ultrasound device calipers. The luteal area and the cavity were measured using ellipse tool, and the area of luteal tissue was obtained by subtracting the area of the cavity of the total luteal area. Using the trace tool, the area of vascularization was defined (Fig. 2), thereby establishing a percentage of vascularized area by dividing the area of vascularization and area of luteal tissue.

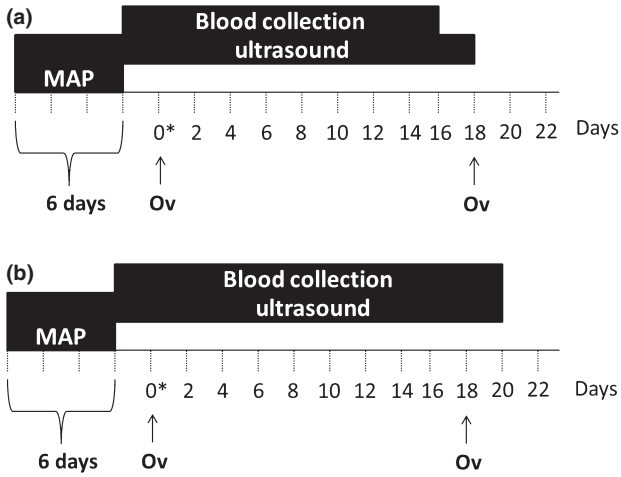

Fig. 1. Schematic representation of the experimental design in non-pregnant (a) and pregnant (b) Santa Inês ewes. *Ovulation occurred approximately at 85 h from sponge removal, and it was considered as Day 0

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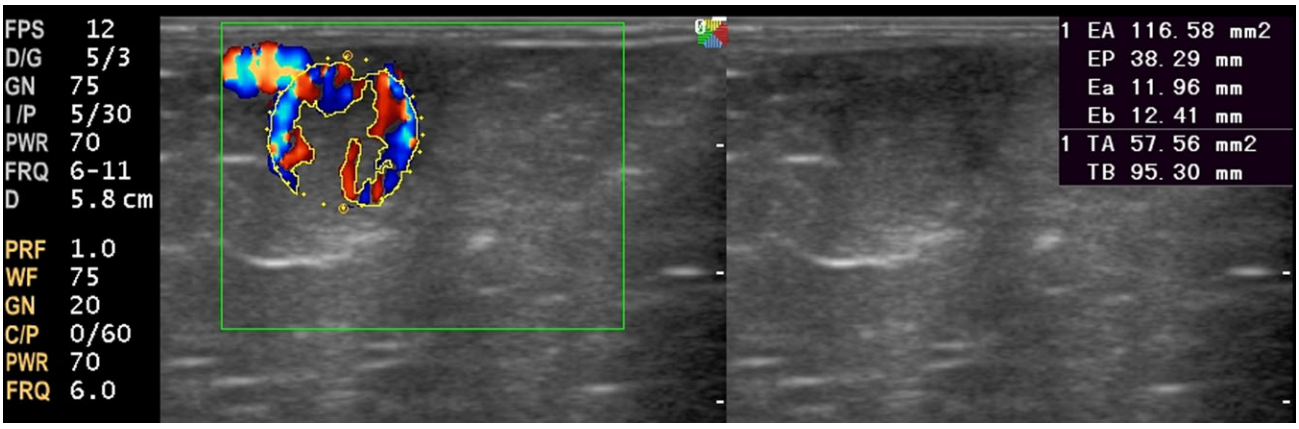

Fig. 2. Dual mode showing simultaneous images at the same time an image of the CL in colour Doppler (left) and B-mode ultrasonography (right). Luteal (EA – ellipse area) and vascularization (TA – trace area) area were calculated in colour Doppler mode. Results are shown in upper right corner

Blood collection and hormonal analysis

Blood was collected once a day in all non-pregnant ewes from the onset of synchronized oestrus to the subsequent oestrus and in pregnant ewes up to day 20, by puncture of the jugular vein into tubes containing EDTA with a vacuum system (Vacutainer©, BD, São Paulo, Brazil). Tubes were immediately placed in ice, transported to the laboratory and centrifuged at 1500 × ***g*** for 15 min. Plasma was removed and stored at −20°C in 1.5 ml tubes pending determination of P_4 concentrations with a commercial solid phase radioimmunoassay (RIA) kit (Coat-a-Count©, Siemens, São Paulo, Brazil) with a sensitivity of 0.2 ng/dl or 0.06 nmol/l in Laboratory of Endocrinology, São Paulo State University, Botucatu – SP (CNEN registration 14 542). The control value was 4.3% and the analytical detection limit was 0.08 ng/ml.

Statistical analysis

Data were analysed according to oestrous cycle period: luteogenesis or luteolysis. Data from all ewes were considered from Day 0 to Day 12 for analysis related to luteogenesis period. For luteolysis period, only data from non-pregnant females were used after Day 13. The onset of luteolysis period was characterized as the time that plasma P_4 concentration abruptly decreased. Data from 48 to 72 h from onset of luteolysis were used for analysis related to luteolysis period. The main effect of time over luteal area, vascularization area and plasma P_4 concentration was evaluated by ANOVA and differences between means compared by Tukey's test. Association between these outcome variables was evaluated by Pearson's correlation method. Analyses were performed using software for statistical analysis SAEG. A probability of $p < 0.05$ indicated that the difference was significant.

Results

Sexual behaviour

A conception rate of 83.3% (10/12) was achieved after natural mating and, thus, a total of eight animals were not pregnant. From these eight ewes, seven repeated oestrus and the duration of oestrous cycle was on average 18.0 ± 0.6 days, and the interval between two ovulations was 17.3 ± 0.8 days. The mean intervals from sponge removal to oestrus were 47.3 ± 19.5 h and to ovulation 85.3 ± 16.9 h. The duration of oestrus was on average 49.3 ± 14.8 h, different ($p < 0.05$) between females with single (38.4 ± 10.0 h) or multiple (53.5 ± 14.4 h) ovulations. The ewes subjected to natural mating presented the duration of oestrus of 47.00 ± 10.8 h, similar ($p > 0.05$) to those only teased (54.00 ± 21.12 h).

Ultrasonography end points and plasma P_4 concentrations

All ovulatory follicles (n = 31) were visualized at the first day of oestrus. Their average diameter was 6.18 ± 1.05 mm (ranging from 4.4 to 8.4 mm). Of 18 ewes, 13 presented multiple ovulations, whereas five showed single ovulation. During the sonogram, on D0 only 32% of the CLs were visualized, on D1 87%, and on D2 all of them were detected. Overall, the CLs were first detected 0.77 ± 0.62 days after ovulation. Although luteal tissue area was greater in animals with multiple ovulations than in those with single ovulation (53.11 ± 10.73 vs 39.35 ± 12.10 mm^2, respectively; $p < 0.05$), the moment of first visualization of the CL was similar (0.46 ± 0.52 vs 1.00 ± 0.70 days after ovulation, $p > 0.05$). Of 31 CL, six (19.4%) presented cavities in the beginning of the evaluations and only three remained visible during the entire experimental period.

No significant differences were found between ewes only teased and those mated in the luteogenesis period. Consequently, animals were viewed as members of one population and data pooled across all 18 ewes. The luteogenesis period was characterized by a progressive increase of luteal area ($p < 0.05$) until D6, and no significant increase was observed on subsequent days (plateau phase) (Fig. 3). The maximum luteal area (124.16 ± 37.78 mm^2) was achieved on D11. The

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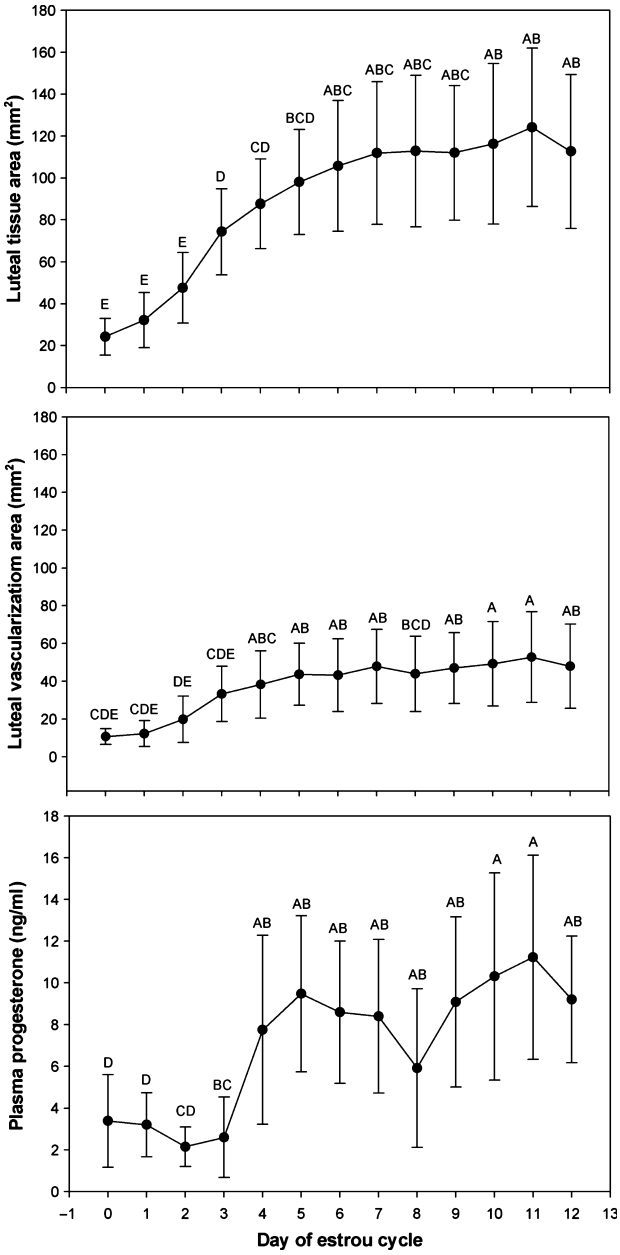

Fig. 3. Changes in luteal tissue area and vascular area of corpora lutea, and plasma progesterone concentrations, observed during luteogenesis in Santa Inês sheep (mean ± SD). [A-E] Values with different superscripts are significantly different $p < 0.05$. Day 0 = Day of ovulation

vascularization area presented increasing growth along with plasma P_4 concentrations, until D4, peaking on D11, 52.78 ± 24.08 mm^2 and 11.23 ± 4.89 ng/ml, respectively (Fig. 3). Synthesis and liberation of P_4 were evidenced by increase in plasma concentrations immediately following ovulation, increasing until the D4 of the oestrous cycle, when they achieved a maximum plateau, which lasts until the moment of luteolysis (Fig. 3). The area of luteal tissue and vascularization were correlated to the P_4 concentrations during luteogenesis ($r = 0.22$ and $r = 0.22$, $p < 0.05$; Fig. 3).

Table 1. Luteal tissue area, plasma progesterone concentration and vascularization area in Santa Inês ewes with single or multiple ovulations on Day 6* (mean ± standard deviation)

Variable	Single ovulation	Multiple ovulations
Repetitions (n)	5	13
CL area (mm^2)	114.00 ± 23.00^a	209.00 ± 52.00^b
Plasma progesterone (ng/ml)	8.02 ± 2.83^a	8.82 ± 3.69^a
Vascularization area (mm^2)	49.00 ± 26.00^a	84.00 ± 27.00^a

[a,b]Values on the same line with different superscripts are significantly different ($p < 0.05$).
*Day 6 = Six days after ovulation.

On D6, when luteal tissue area reached its maximum increase, its values were greater in animals with multiple ovulations when compared to those with single ovulation (209.00 ± 52.00 vs 114.0 ± 23.00 mm^2, respectively, $p < 0.05$). Despite the greater luteal tissue area in animals with multiple ovulations, no differences in vascularization area and plasma P_4 concentration were observed when compared to animals with single ovulation (Table 1).

The luteolysis period was characterized by an abrupt decrease in plasma P_4 concentrations, reaching values < 1.0 ng/ml 24 h after the onset of luteolysis in non-pregnant ewes. Differently, luteal tissue area and vascularization area decreased gradually, with significant ($p < 0.05$) decrease observed, respectively, 48 and 24 h after the onset of luteolysis (Figs 4 and 5). Correlation between P_4 concentrations and luteal tissue area ($r = 0.41$, $p < 0.05$) and between P_4 concentrations and vascularization area ($r = 0.48$, $p < 0.05$) was also observed during luteolysis. Interestingly, it was possible to detect the CL from the earlier oestrous cycle at the moment of the next ovulation. The colour Doppler was an efficient tool to distinguish the CL which was in regression and the one that is developing.

Discussion

As expected, luteal dynamics was characterized by three distinctive phases: a progressive increase of luteal tissue area (luteogenesis), followed by a plateau phase and finally a decrease of luteal tissue area (luteolysis). The patterns of the luteal dynamics observed in Santa Inês breed in the present study were similar to those observed for other sheep breeds (Bartlewski et al. 1999; Gonzalez-Bulnes et al. 2000; Davies et al. 2006; Contreras-Solis et al. 2008) and other domestic ruminants, such as caprine (Arashiro et al. 2010a) and bovine (Kastelic et al. 1990; Siqueira et al. 2009).

During luteogenesis period, to synthesize P_4, an intense cellular proliferation and biochemical changes are observed in the CL (Smith et al. 1994; Sangha et al. 2002). Corroborating with these findings and previous studies in sheep (Davies et al. 2006), cows (Siqueira et al. 2009) and goats (Arashiro et al. 2010a), a positive correlation between luteal tissue area and plasma P_4

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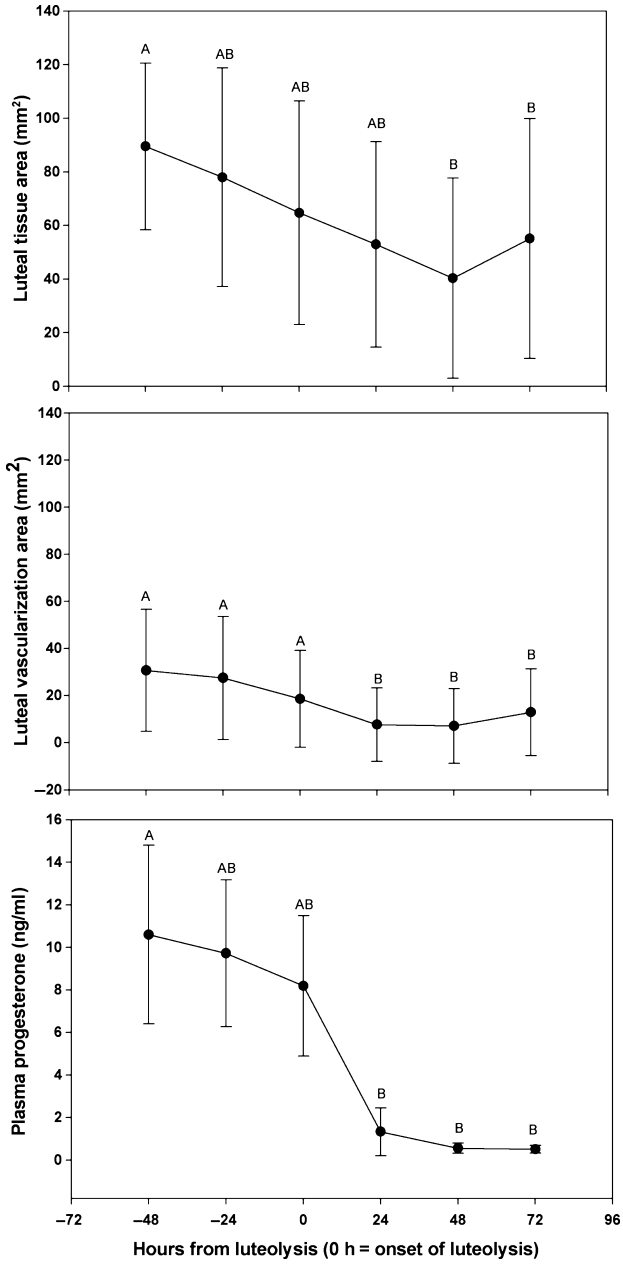

Fig. 4. Changes in luteal tissue area and vascular area of corpora lutea, and plasma progesterone concentrations, observed during luteolysis in Santa Inês sheep (mean ± SD). ^{AB} Values with different superscripts are significantly different $p < 0.05$

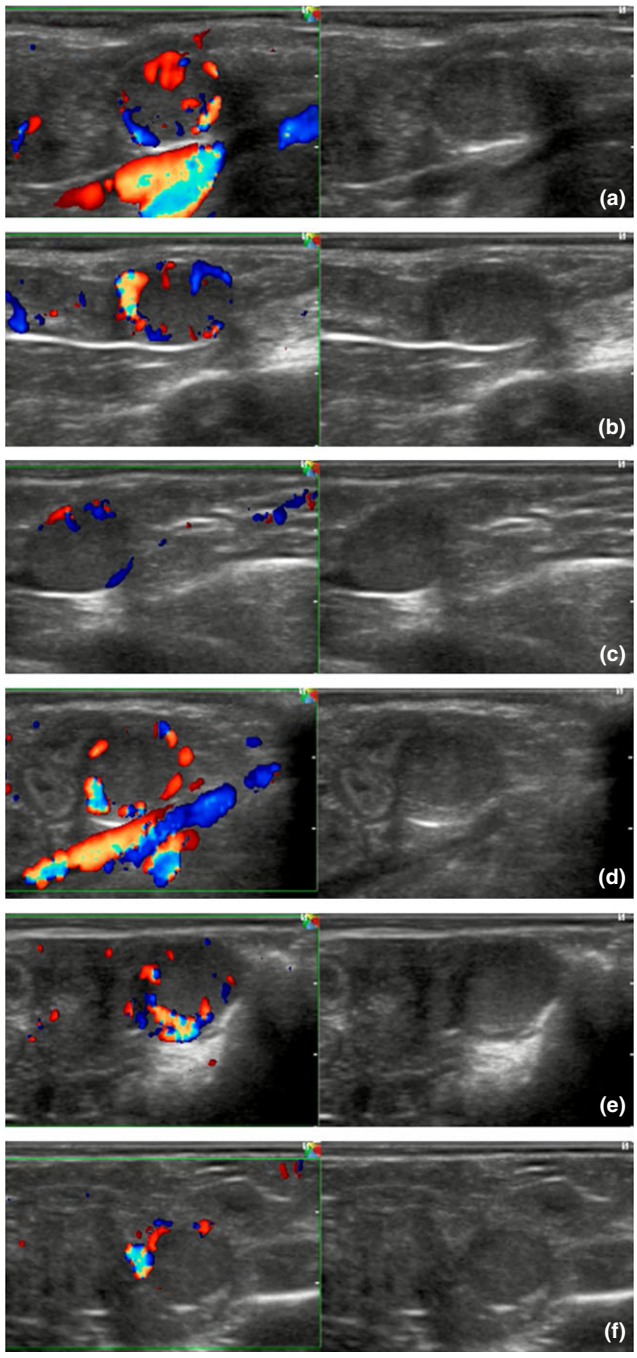

Fig. 5. Dual mode images showing simultaneous images of a corpus luteum in colour Doppler (left) and B-mode ultrasonography (right) at different times relative to luteolysis. (a) −48 h, (b) −24 h, (c) 0 h – onset of luteolysis, (d) +24 h, (e) +48 h and (f) +72 h

concentration was observed during luteogenesis. These studies in ruminants also observed this positive correlation during luteolysis. However, P_4 concentrations decreased abruptly, while luteal tissue area gradually decreased. According to McCraken et al. (1999), the luteolysis process is characterized initially by a loss in steroidal capacity (functional luteolysis) followed by a luteal tissue regression (structural luteolysis). Indeed, structural luteolysis requires more time once this process involves an apoptotic cascade (luteal cell death and phagocytosis) and replacement by fibroblasts (Niswender et al. 2000), leading to corpus albicans formation.

Although the P_4 produced by functional CL has an important role in establishment and maintaining pregnancy (Niswender et al. 2000), the results in Table 1 demonstrate that the ability for synthesis and release of P_4 was not different between ewes with one or two

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ovulations. Bartlewski et al. (1999) observed that the mechanisms that control the formation of luteal tissue and P_4 secretion in ewes seem to be breed specific, differing between prolific and non-prolific ewes. Other results in goats (Fonseca and Torres 2005) suggest that the maximum plasma concentration of P_4 is a breed characteristic and it is not associated with the number of CL. It was observed that prolific ewes had higher ovulation rate, resulting in smaller CL and lower P_4 production than non-prolific ewes (Bartlewski et al. 1999).

The presence of a functional vascular network in an organ or a specific structure (e.g. ovarian follicle or CL) is important to develop their biological role. After ovulation, the development of CL is not only characterized by an intense cellular and biochemical changes (Smith et al. 1994; Sangha et al. 2002), but also by an intense process of angiogenesis (reviewed by Hazzard and Stouffer 2000). In the present study, luteal vascularization progressively increased until D4. This result is in agreement with a previous study in bovine, which reported that angiogenesis achieved maximum growth in CL between the second and third day after ovulation (Reynolds et al. 2000). Moreover, the increase observed in luteal vascularization during luteogenesis period was associated with the increase in luteal area and plasma P_4 concentration. The presence of a functional vascular system in the CL provides oxygen, nutrients, hormones and other factors necessary to luteal growth and steroidogenesis. This result demonstrates that assessment of luteal vascularization by colour Doppler US has the potential to be used as a parameter to evaluate luteal function in sheep. Similar results were previously observed in bovine (Acosta et al. 2003) and buffaloes (Russo et al. 2010).

The association between luteal vascularization and luteal function was also observed during luteolysis period. In addition to plasma P_4, luteal vascularization area decreased after onset of luteolysis. A previous study in cows with PGF-induced luteolysis (Acosta et al. 2002) demonstrated that changes in the vascular parameters of the CL could be detected 24 h after the onset of luteolysis, similar to the current study using sheep with natural luteolysis. The decrease in luteal vascularization, as well as the decrease in P_4 concentration, occurred faster than the decrease in luteal tissue area. This effect also was observed by Herzog et al. (2010), which concluded that blood flow is a more appropriate indicator for luteal function that luteal size, specifically in the luteal regression in cows. This temporal difference between functional and structural luteolysis can lead the technician to errors when evaluating an early regressing CL using only B-mode ultrasonography. The premature luteal regression is very common in small ruminants in D3–D6 after the onset of oestrus, mainly in superovulated animals (revised by Saharrea et al. 1998). The use of colour Doppler US can overcome this difficulty, as a reduced, or even the absence of blood flow can be detected 24 h after onset of luteolysis, while changes in morphological parameters will occur later, demonstrating that the colour Doppler mode can be an important tool in the evaluation and selection of embryo recipients for non-surgical transfer.

Besides functional evaluation of the CL, the colour Doppler US showed to be a valuable tool to identify the ovaries and its structures. In small ruminants, the identification of the ovaries and its structures using B-mode ultrasonography is a challenge (Bicudo et al. 2009), especially for inexperienced technicians. The assessment of vascularization by colour Doppler helped identifying the CL during its initial development phases. Previous studies using B-mode US have demonstrated that CL was first detected between day 2 and day 5 of the oestrous cycle (Orita et al. 2000; Riesenberg et al. 2001; Medan et al. 2004; Simões et al. 2007; Arashiro et al. 2010b). In the present study, CL was first detected in the first day of the oestrous cycle. This early detection of CL's in colour Doppler mode was facilitated because after ovulation, the angiogenesis is already present, due to increased blood flow area in ovulatory follicles (El-Sherry et al. 2013).

Conclusion

The luteal dynamics of Santa Inês sheep showed patterns similar to those observed in other sheep breeds studied. The colour Doppler ultrasonography allowed the identification of the CL one day after ovulation and it has the potential to be used as a tool to assess luteal function in sheep.

Acknowledgements

This research was supported by Faperj (E-26/102.963/2012), Universidade Federal Fluminense/Proppi/Fopesq, and Embrapa. JFF, JMGSF, EO and FZB are CNPq fellows.

Conflict of interest

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

Author contributions

JF Fonseca, EKN Arashiro, JHM Viana and FZ Brandão contributed to the design of the study. LM Figueira, EKN Arashiro and ACS Ribeiro collected the samples. E. Oba was responsible for the progesterone concentrations analysis. LM Figueira, EKN Arashiro and JMG Souza-Fabjan analysed the data. LM Figueira, EKN Arashiro and JMG Souza-Fabjan contributed to the draft. JF Fonseca, JHM Viana and FZ Brandão approved the final version to be published.

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References

Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A, 2002: Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F2 alpha injection in the cow. Biol Reprod **66**, 651–658.

Acosta TJ, Hayashi KG, Ohtani M, Miyamoto A, 2003: Local changes in blood flow within the preovulatory follicle wall and early corpus luteum in cows. Reproduction **125**, 759–767.

Arashiro EK, Fonseca JF, Siqueira LGB, Fernandes CA, Brandão FZ, Oba E, Viana JH, 2010a: Assessment of luteal function in goats by ultrasonographic image attribute analysis. Small Rumin Res **94**, 176–179.

Arashiro EK, Viana JHM, Fonseca JF, Camargo LSA, Fernandes CAC, Brandão FZ, 2010b: Luteal dynamics in goats: morphological and endocrine features. R Bras Zootec **39**, 1937–1942.

Bari F, Khalid M, Haresign W, Murray A, Merrel B, 2003: Factors affecting the survival of sheep embryos after transfer within a MOET program. Theriogenology **59**, 1265–1275.

Bartlewski PM, Beard AP, Rawlings NC, 1999: An ultrasonographic study of luteal function in breeds of sheep with different ovulation rates. Theriogenology **52**, 115–130.

Bicudo SD, Biscarde CEA, Monteiro CD, Bittencourt RF, Avelar SR, Oliveira TM, Rodello L, Cesar CNR, Oba E, 2009: The use of metallic implants to facilitate follicular dynamics study in ewes. Anim Reprod **6**, 253 (Abstract).

Bollwein H, Meyer HH, Maierl J, Weber F, Baumgartner U, Stolla R, 2000: Transrectal Doppler sonography of uterine blood flow. Theriogenology **53**, 1541–1552.

Bollwein H, Baumgartner U, Stolla R, 2002: Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. Theriogenology **57**, 2053–2061.

Contreras-Solis I, Diaz T, Lopez G, Caigua A, Lopez-Sebastian A, Gonzalez-Bulnes A, 2008: Systemic and intraovarian effects of corpus luteum on follicular dynamics during estrous cycle in hair breed sheep. Anim Reprod Sci **104**, 47–55.

Davies KL, Bartewski PM, Pierson RA, Rawlings NC, 2006: Computer-assisted image analyses of corpora Luteal in relation to peripheral concentrations of progesterone: a comparison between breeds of sheep with different ovulation rates. Anim Reprod Sci **96**, 165–175.

Domínguez E, Rivera Del Álamo MM, Novellas R, Espada Y, Santos L, García F, Andaluz A, Fresno L, 2013: Doppler evaluation of the effects of propofol, etomidate and alphaxalone on fetoplacental circulation hemodynamics in the pregnant ewe. Placenta **34**, 738–744.

El-Sherry TM, Derar R, Bakry R, 2013: Changes in blood flow in ovine follicles and serum concentration of estradiol 17 beta (E2) and nitric oxide (NO) around the time of ovulation in Ossimi ewes. Anim Reprod Sci **138**, 188–193.

Fonseca JF, Torres CAA, 2005: Administration of hCG 5 days after breeding and reproductive performance in nulliparous dairy goats. Reprod Domest Anim **40**, 495–499.

Ginther OJ, Silva LA, Araujo RR, Beg MA, 2007: Temporal associations among pulses of 13,14-dihydro-15-keto-PGF2alpha, luteal blood flow, and luteolysis in cattle. Biol Reprod **76**, 506–513.

Gonzalez-Bulnes A, Santiago-Moreno J, Gomez-Brunet A, Lopez-Sebastian A, 2000: Relationship between ultrasonographic assessment of the corpus luteum and plasma progesterone concentration during the oestrous cycle in monovular ewes. Reprod Domest Anim **35**, 65–68.

Griffin PG, Ginther OJ, 1992: Research applications of ultrasonic imaging in reproductive biology. J Anim Sci **70**, 953–972.

Hazzard TM, Stouffer RL, 2000: Angiogenesis in ovarian follicular and luteal development. Best Pract Res Clin Obstet Gynaecol **14**, 883–900.

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.

Honnens A, Voss C, Herzog K, Niemann H, Rath D, Bollwein H, 2008: Uterine blood flow during the first 3 weeks of pregnancy in dairy cows. Theriogenology **70**, 1048–1056.

Kastelic JP, Pierson RA, Ginther OJ, 1990: Ultrasonic morphology of corporal lutea and central Luteal cavities during the estrous cycle and early pregnancy in heifers. Theriogenology **34**, 487–498.

Marret H, Brewer M, Giraudeau B, Tranquart F, Satterfield W, 2006: Assessment of cyclic changes of microvessels in ovine ovaries using Sonovue contrast-enhanced ultrasound. Ultrasond Med Biol **32**, 163–169.

McCraken JA, Custer EE, Lamsa JC, 1999: Luteolysis: a neuroendocrine-mediated event. Physiol Rev **79**, 263–304.

Medan MS, Watanabe G, Sasaki K, Taya K, 2004: Transrectal ultrasonic diagnosis of ovarian follicular cysts in goats and treatment with GnRH. Domest Anim Endocrinol **27**, 115–124.

Menzies PI, 2007: Reproductive health management programs. In: Youngquist RS, Threlfall WR (eds), Current Therapy in Theriogenology. 2nd edn. Saunders Elsevier, Phyladelphia, pp. 701–714.

Miller BG, Moore NW, 1976: Effects of progesterone and oestradiol on RNA and protein metabolism in the genital tract and on survival of embryos in the ovariectomized ewe. Aust J Biol Sci **29**, 565–573.

Miyamoto A, Shirasuna K, Hayashi KG, Kamada D, Awashima C, Kaneko E, Acosta TJ, Matsui MA, 2006: Potential use of color ultrasound as a tool for reproductive management: New observations using color ultrasound scanning that were not possible with imaging only in black and white. J Reprod Dev **52**, 153–160.

Niswender GD, Juengel LJ, Silva PJ, Rollyson MK, McIntush EW, 2000: Mechanism controlling the function and life span of the corpus luteum. Physiol Rev **80**, 1–29.

National Research Council – NRC, 2007: Nutrient Requirements of Small Ruminants. National Academy Press, Washington, DC.

Oliveira M, Feliciano M, D'Amato C, Oliveira L, Bicudo S, Fonseca J, Vicente W, Visco E, Bartlewski P, 2014: Correlations between ovarian follicular blood flow and superovulatory responses in ewes. Anim Reprod Sci **144**, 30–44.

Orita J, Tanaka T, Kamomae H, Kaneda Y, 2000: Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. J Reprod Dev **46**, 31–37.

Panarace M, Garnil C, Cané L, Rodriguez E, Medina M, 2008: EchoDoppler ultrasonographic assessment of resistance and velocity of blood flow in the ductus venosus throughout gestation in fetal lambs. Theriogenology **70**, 648–654.

Pieterse MC, Taverne MAM, Kruip AM, Willemse AH, 1990: Detection of corpora lutea and follicles in cows: a comparison of transvaginal ultrasonography and rectal palpation. Vet Rec **126**, 552–554.

Reynolds LP, Grazul-Bilska AT, Redmer DA, 2000: Angiogenesis in the corpus luteum. Endocrine **12**, 1–9.

Riesenberg S, Meinecke-Tillmann S, Meinecke B, 2001: Ultrasonic survey of follicular development following superovulation with a single application of FSH, eCG or hMG in goats. Small Rumin Res **40**, 83–93.

Russo M, Vecchio D, Neglia G, Pacelli C, Prandi A, Gasparrini B, Zicarelli L, D'Occhio MJ, Campanile G, 2010: Corpus luteum function and pregnancy outcome in buffaloes during the transition period from breeding to non-breeding season. Reprod Domest Anim **45**, 988–991.

Saharrea A, Valencia J, Balcázar A, Mejía O, Cerbón JL, Caballero V, Zarco L, 1998: Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. Theriogenology **50**, 1039–1052.

Sangha GK, Sharma RK, Guraya SS, 2002: Biology of corpus luteum in small ruminants. Small Rumin Res **43**, 53–64.

Siddiqui MA, Almaumun M, Ginther OJ, 2009: Blood flow in the wall of the preovulatory follicle and its relationship to pregnancy establishment in heifers. Anim Reprod Sci **113**, 287–292.

Simões J, Almeida JC, Valentim R, 2007: Assessment of luteal function by ultrasonographic appearance and measurement

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of corpora lutea in goats. Anim Reprod Sci **97**, 36–46.

Singh J, Adams GP, Pierson RA, 2003: Promise of new image technologies for assessing ovarian function. Anim Reprod Sci **78**, 371–399.

Siqueira LGB, Torres CAA, Amorim LS, Souza ED, Camargo LSA, Fernandes CAC, Viana JHM, 2009: Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle. Anim Reprod Sci **115**, 18–28.

Siqueira LGB, Areas VS, Ghetti AM, Fonseca JF, Palhão MP, Fernandes CAC, Viana JHM, 2013: Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. J Dairy Sci **96**, 6461–6472.

Smith MF, McIntush EW, Smith GW, 1994: Mechanism associated with corpus luteum development. J Anim Sci **72**, 857–872.

Utt MD, Johnson GL, Beal WE, 2009: The evaluation of corpus luteum blood flow using color-flow Doppler ultrasound for early pregnancy diagnosis in bovine embryo recipients. Theriogenology **71**, 707–715.

Wilmut I, Sales DI, Ashworth CJ, 1985: The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. Theriogenology **23**, 107–119.

Submitted: 3 Feb 2015; Accepted: 25 Apr 2015

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