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Accuracy of assessment of luteal morphology and luteal blood flow for prediction of early pregnancy in goats

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ABSTRACT

This study aims to determine the best post-breeding time for an early pregnancy diagnosis in dairy goats, when using luteal morphology and vascularization assessment by B-Mode and color Doppler ultrasonography (US) in association or not with the anechoic uterine content. A total of 131 Saanen goats (2.0 ± 0.5 years old) were used in the two studies. In the first study, pregnancy prediction was daily performed from Day 15–23 post-breeding in 51 does. This was based on a subjective assessment of luteal morphology (B-Mode US), luteal blood flow (color Doppler US), and the presence of anechoic uterine content (B-Mode US). In the second study, pregnancy predictions were performed in 71 does on the best post-breeding day, as determined in the first study (Day 21 using luteal blood-flow assessment and Day 23 using luteal morphological assessment and overall analysis). In both studies, pregnancy diagnosis was confirmed on Day 30 (gold standard method by B-Mode transrectal ultrasonography). The B-Mode and color Doppler US performance in reaching an early pregnancy diagnosis was evaluated by calculating sensitivity (Sens), specificity (Spec), negative and positive predictive values (NPV and PPV), accuracy (Acc), the Kappa index (κ), and the Younden index (J). In Study 1, the pregnancy prediction accuracy progressively increased from Day 17–23 using B-Mode US (D17: 50.98%; D18: 52.94%; D19: 62.75%; D20: 74.51%; D21: 86.27%; D22: 90.20%; D23: 96.08%), and from Day 17–21 using color Doppler US (D17: 49.02%; D18: 54.90%; D19: 70.59%; D20: 86.27%; D21: 96.08%). In the second study, color Doppler, B-Mode, and overall assessments at Day 21 and Day 23, respectively, presented a similar pattern of sensitivity, specificity, and accuracy as was found in the first study. Therefore, both color Doppler and B-Mode assessments can be judged as effective tools for reaching a pregnancy diagnosis in goats as early as on Day 21 and Day 23, respectively, post-breeding.

1. Introduction

An early and accurate pregnancy diagnosis is essential for optimizing reproductive performance in ruminants [1–3]. Different techniques have been investigated for this purpose, including ultrasonography (US), rectal/abdominal palpation, pregnancy-specific proteins, hormonal assay, and X-ray techniques [1–3]. Overall, when comparing these distinct techniques, the transrectal US is the simplest and less invasive, while achieving a prompt outcome [1–3].

As revised by Freitas and Simplício [3], the ultrasound pregnancy diagnosis in goats was initially applied to aim for a better accuracy through abdominal exams using A-Mode (50–120 days post-breeding), B-Mode (40–75 days post-breeding) or Doppler Effect (75–150 days post-breeding). Nevertheless, as early as 30 days after mating, it was possible to confirm pregnancy by uterine findings, such as uterine distention, anechoic content, embryonic vesicle, and embryonic heartbeat by transrectal B-Mode US (associated or not with Doppler).

In the light of studies carried out in cattle [4,5], the luteal blood flow assessment by Doppler US has been proposed to achieve an

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earlier pregnancy diagnosis in small ruminants and to reduce the artificial insemination (AI) or mating interval when associated with a resynchronization protocol. Preliminary studies reported restricted relations among the luteal tissue area, luteal blood flow, and plasma P4 throughout the estrous cycle, mainly at the luteal static and growth phase [6–9]. However, at the luteolysis phase, the luteal blood flow was more sensible, allowing for the detection of minor changes in the P4 values when compared with the luteal tissue area [6–9]. From a physiology perspective, these findings can be justified by the luteolysis phase and its functional and structural steps [6–9]. As the early pregnancy diagnosis occurs close to the luteolysis phase, the luteal blood flow could be a better candidate for providing information regarding pregnancy status. Despite the accuracy, previous studies analyzed the luteal blood flow and B-Mode images using a software program, which requires some extra time to reach an outcome and is also complicated to apply under field conditions [6–9].

A recent study proposed a subjective score scale for the luteal blood flow (scale 1–4) in ewes, after mating, for early pregnancy diagnosis on the 17th day of the estrous cycle [10]. Besides, a positive relation was found among luteal score scales between evaluators, showing that this technique could be accurate (85.7%), repeatable (icc = 0.95), with early (Day 17) and real-time results [10]. In addition, Balaro et al. [9] also found an agreement between the subjective and pixel analysis of the luteal blood flow throughout the whole luteal phase ($k = 0.65$; $P < 0.05$). In this sense, the subjective assessment can be perfectly adapted to evaluate the luteal blood flow.

Although some recent studies have explored luteal blood flow potential for early pregnancy diagnosis, none of them have considered a subjective assessment of the luteal morphology (echogenicity and echotexture) using B-Mode US for the same purpose [6,7,9]. Indeed, this was only found in a study regarding pixel analysis of the luteal morphology in cattle to determine luteolysis [11]. Thus, studies on this subject have not been proposed in goats until recently.

To determine the earliest post-breeding time for reaching an early pregnancy diagnosis in goats, this study aims to evaluate the accuracy of: (1) the subjective luteal blood flow assessment using color Doppler US, (2) the subjective luteal morphology assessment using B-Mode US, (3) the presence of anechoic uterine content, and (4) a validation of its use in dairy goat farm management.

2. Materials and methods

2.1. Experimental location, design, and animals

This two-step study was conducted in a dairy goat farm located in Rio de Janeiro state, Brazil (22°07′50.2″S, 42°47′47.1″W) during the nonbreeding season (Nov/2017–Feb/2018). According to Köppen [12], the local climate is a tropical hot-humid type (Aw). In the first study (pilot), the accuracy of B-Mode and color Doppler US as tools for early pregnancy diagnosis was evaluated from Day 15 to Day 23 post-AI, as previously suggested by Balaro et al. [9]. After determining the best moment post-breeding for the early pregnancy diagnosis, a second study (validation data) was designed to evaluate the application of B-Mode and color Doppler US in order to determine their practicality as pregnancy diagnosis tools. For both studies, 131 Saanen goats {2.0 ± 0.5 years old, body condition score: 3.0 ± 0.2 (scale 1–5 [13]) (mean ± SD)} were used. All animals were previously submitted to a gynecological exam and only does without reproductive abnormalities detected by US or clinical exam were used. Moreover, all goats were considered in the anestrus time due to the absence of a corpus luteum (CL) in the US. Throughout the study, does were kept confined in collective pens and were fed twice a day with corn silage, alfalfa hay (*Medicago sativa*), and concentrate, according to their maintenance requirements (16% crude protein [14]). Water and mineral salt for goats (Caprinofós, Tortuga, São Paulo, Brazil) were provided *ad libitum*.

All procedures performed in this study were previously approved by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (protocol 1021) and were carried out under the ethical principles of the Sociedade Brasileira de Experimentação Animal. Moreover, this manuscript follows the guidelines of *Animal Research: Reporting of In Vivo Experiments* [15].

2.2. Study 1

2.2.1. Estrus synchronization and artificial insemination (AI)

Estrus synchronization protocol and artificial insemination were performed in 60 animals as follows: an intravaginal sponge impregnated with 60 mg of medroxyprogesterone acetate (Progespon®, Schering Plough, São Paulo, Brazil) was inserted and kept for 11 days. Twenty-four hours before sponge removal, all animals received 0.125 mg of cloprostenol sodium (Estron®, Agner União, São Paulo, Brazil) and 200 IU of equine chorionic gonadotropin (Novormon®, Schering Plough, São Paulo, Brazil). The intervals between sponge removal and AI were calculated according to the moment of estrus behavior presentation (detected by a male) after sponge removal [16]. Animals with estrus behavior between 12 and 24 h after sponge removal were inseminated after 36 h had elapsed. Those with estrus manifestation at 36 h after sponge removal were inseminated 12 h later. Animals with estrus behavior between 48 and 60 h after sponge removal and those without estrus behavior were inseminated 60 h after sponge removal. The AI technique was performed in all females as described by Fonseca et al. [17]. Briefly, the goats were contained in the milking room in a standing position. A lubricated Collin-type speculum was used (in sizes 1 or 2, according to the goat) to enter the vulva and vagina, and facilitate the visualization of the cervical ostium projected into the speculum. An Allis clamp was used to immobilize the cervix, then the inseminator introduced the semen applicator into the cervical orifice, making lateral–lateral and cranio–ventral movements with light and forward cranial pressure to pass through the cervical path, which was stopped when loss of resistance was detected, indicating the entrance inside the uterus. At this point, approximately 300×10^6 sperm (0.25 mL) of fresh semen from fertile bucks of the same flock was deposited into the uterus. All goats were inseminated once and in the same day (Day 0), even if the time of the day was calculated according to the beginning of estrus behavior (morning and evening). Estrus behavior was no longer seen among all animals 72 h after sponge removal, indicating that ovulation had occurred and, therefore, this was considered the first day of the estrous cycle (Day 1).

2.2.2. Sonograms and early pregnancy prediction

Ultrasonographic assessments of the ovaries were performed every 24 h, from Day 15 to Day 23, using portable equipment (Sonoscape S6, Shenzhen, China) with a 7.5 MHz linear rectal transducer adapted to be used in small ruminants. Initially, the reproductive tract was scanned using B-Mode US to locate the ovaries bearing one or more CL. At this point, nine goats did not present a CL, and were not evaluated from D16 on.

A morphological luteal assessment was performed using a subjective score scale (ranging from 1 to 3) according to the echogenicity and echotexture; briefly 1: near anechoic, heterogeneous, and coarse granulation, 2: hypoechoic, homogeneous, and fine granulation and 3: echogenic, homogeneous, and fine granulation (Fig. 1) adapted from Simões et al. [18]. Animals bearing at

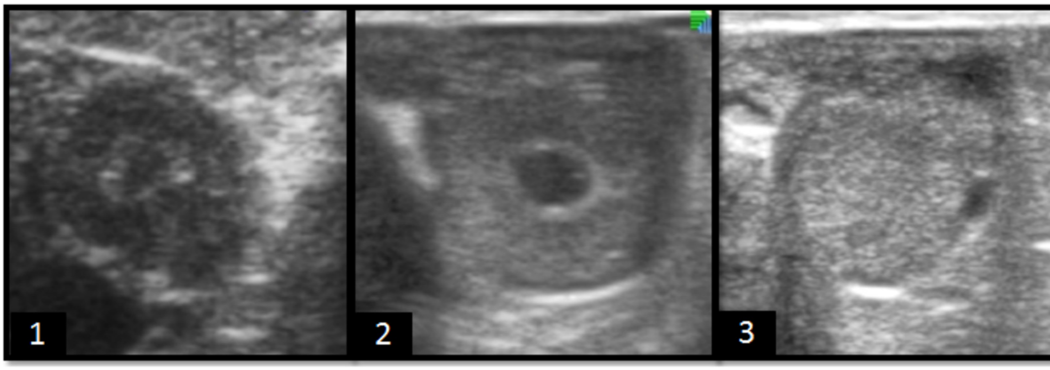

Fig. 1. Ultrasonographic images demonstrating the score scale used for the subjective luteal morphological assessment: 1 (near anechoic, heterogeneous, and coarse granulation), 2 (hypoechoic, homogeneous, and fine granulation) and 3 (echogenic, homogeneous, and fine granulation).

least one CL, with or without a cavity, with a score ≥2 were considered as pregnant.

Thereafter, color flow mode (CFM) was activated, and a complete scan of the CL was performed. Luteal blood flow was evaluated using a subjective score scale ranging from 1 to 4 – briefly, 1: 0–25%, 2: 26–50%, 3: 51–75%, and 4: 76–100% – as proposed by Arashiro et al. [10] (Fig. 2). Animals bearing at least one CL with a blood-flow score ≥2 were considered pregnant.

All does were also evaluated for the presence of anechoic uterine content from Day 15 to Day 23 (adapted from Martinez et al. [19]). In addition, to improve the early pregnancy diagnosis accuracy, whether there was any association between the uterine content and the presence of a positive CL on B-Mode and color Doppler was also analyzed. To achieve this aim, the accuracy evaluations were organized in three different ways: (1) the presence of anechoic uterine content being considered as positive; (2) the presence of anechoic uterine content with a positive CL on B-Mode assessment to be considered as positive; and (3) the presence of anechoic uterine content with a positive CL on color Doppler assessment to be considered as positive. The (2) and (3) evaluations were considered representative of the overall performance (uterine + luteal assessment) of the US test.

Pregnancy was confirmed on Day 30 (gold-standard) based on the detection of at least one viable embryonic vesicle (embryo with heartbeats) within the uterine horn using B-Mode US. All exams were performed by the same technician and B-Mode and color Doppler settings were standardized and maintained constant throughout the experiment period (Frames *per* second [FPS]: 23, Depth [D]: 6.7 cm, Gain [GN]: 255, CFM frequency: 6.0 MHz, Pulse Repetition Frequency [PRF]: 1.0 KHz, and Wall Filter [WF]: 75 KHz).

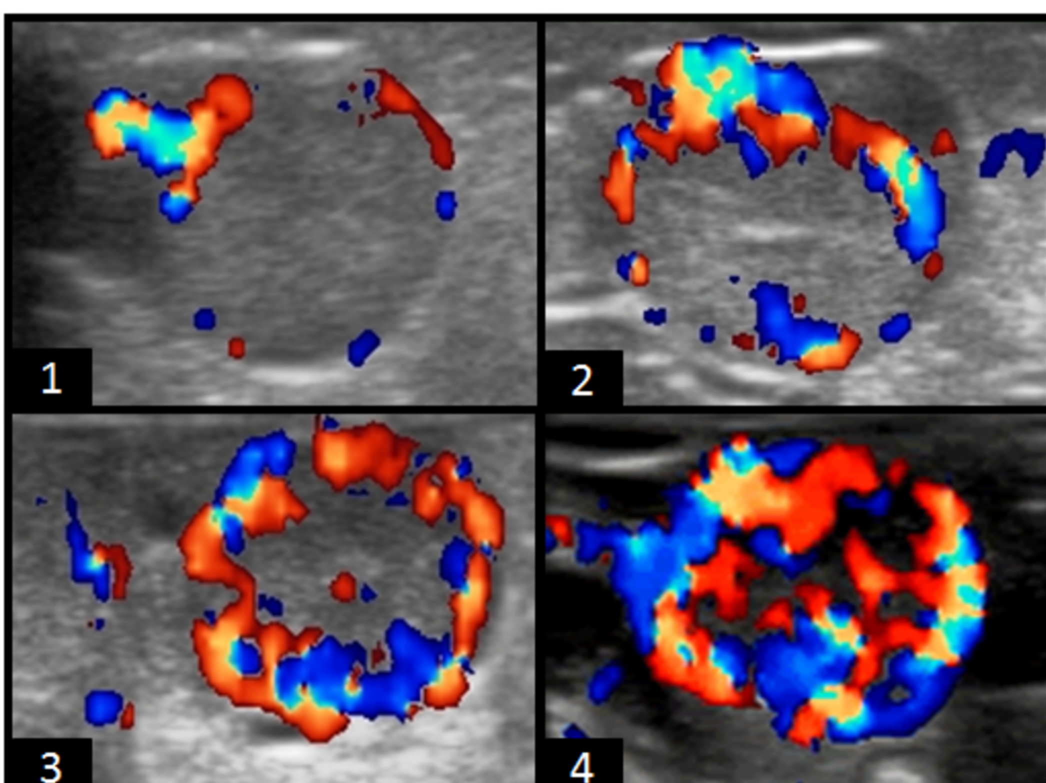

Fig. 2. Ultrasonographic images demonstrating the score scale used for subjective luteal blood-flow assessment: 1 (0–25%), 2 (26–50%), 3 (51–75%) or 4 (76–100%).

Pregnancy predictions by uterine content echogenicity, luteal morphology, luteal blood flow, and overall performance, from Day 15 to Day 23, were compared to outcomes obtained on Day 30 and classified as follows: true positive (TP; animals diagnosed as pregnant by both methods), true negative (TN; animals diagnosed as non-pregnant by both methods), false positive (FP; animals diagnosed as pregnant by luteal morphology, luteal blood-flow assessment or overall assessment, but as non-pregnant by B-Mode US on Day 30), and false negative (FN; animals diagnosed as non-pregnant by luteal morphology, luteal blood-flow assessment or overall assessment, but as pregnant by B-Mode US on Day 30).

2.3. Study 2

2.3.1. Estrus synchronization and AI

In this study, 71 different does were submitted to the same estrus synchronization protocol described in Study 1 and AI was performed between 45 h and 49 h after sponge removal, using the same technique referred to in item 2.2.1.

2.3.2. Sonograms and early pregnancy prediction

Ultrasonographic observations were performed according to the methodology described in Study 1. In addition, it was only performed at the best days as defined by the first study (Day 21 using luteal assessment by color Doppler US; Day 23 using luteal assessment B-Mode US, and B-Mode overall assessment).

2.4. Data analysis

In Study 1, the overall performance of the subjective luteal assessment from Day 15 to Day 23 and the agreement between color Doppler and B-Mode US with the outcome on Day 30 (gold-standard) was classified using Kappa index (κ) and the following parameters: Sensitivity (Sens), Specificity (Spec), Positive and Negative Predictive Value (PPV and NPV, respectively), Accuracy (Acc), positive and negative Likelihood Ratio (LR+ and LH-, respectively), and Younden index (J). The equations used to calculate each parameter were as follows: Sens = TP/(TP + FN); Spec = TN/(FP + TN); PPV = TP/(TP + FP); NPV = TN/(FN + TN); Acc = (TP + TN)/n; LR+ = Sens/100-Spec; LR- = 100-Sens/Spec; J = Sens + Spec − 1. Furthermore, the Fisher Exact and Qui-square tests were performed to compare the frequencies of FP and Acc between B-Mode and color Doppler US assessments over time, as well as to compare the frequencies of Acc between B-Mode, color Doppler US and overall assessments at a level of 5% of significance.

The same parameters were also calculated in Study 2 to determine the efficiency of the subjective luteal blood flow, luteal morphology assessment, and presence of anechoic uterine content at Day 21 and 23, respectively, for early pregnancy diagnosis.

3. Results

3.1. Study 1

The overall performance of the subjective luteal assessment using B-Mode and color Doppler US as a tool for early pregnancy diagnosis, from the 15th Day to the 23rd Day post-insemination, is presented in Table 1 and Fig. 3. Pregnancy diagnosis by subjective luteal assessment using B-Mode and color Doppler US on Days 15 and 16 were demonstrated to be unfeasible. During this period, all CL were considered active and, consequently, all animals diagnosed as non-pregnant animals on Day 30 were classified as pregnant. From Day 17–23, the number of FP and TN results progressively reduced and increased, respectively, until Day 23 using B-Mode, while the color Doppler assessment reached its maximum on Day 21 and did not change until Day 23. Two animals diagnosed as non-pregnant on Day 30 had an echogenic and vascularized CL (score ≥ 2) until Day 23. Only one FN result was observed in this study (Day 16 using color Doppler US). However, this FN result did not persist on subsequent days.

The presence of anechoic uterine content assessment is presented in Table 2. The uterine content echogenicity showed FP from Day 19–21, which did not happen when the evaluations associated with the CL analysis were made. Moreover, there was no difference between the uterine content echogenicity associated with B-Mode and the one associated with color Doppler.

When comparing the efficiency of B-Mode and color Doppler assessments, there was no difference in the distribution of FP frequencies and accuracy within experimental days between tests (Fig. 3).

Table 1
Subjective luteal morphological assessment using B-Mode US and subjective luteal blood-flow assessment using color Doppler US for early pregnancy diagnosis in dairy goats (n = 51) from Days 15–23 (post-insemination) in Study 1.

Day of the cycle	TP, FP, FN, TN (n)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Acc (%)	κ	Youden's index
				B-Mode				
D15	25,26,0,0	100	0.00	49.02	NC	49.02	0.00	0.00
D16	25,26,0,0	100	0.00	49.02	NC	49.02	0.00	0.00
D17	25,25,0,1	100	3.85	50.00	100	50.98	0.04	3.85
D18	25,24,0,2	100	7.69	51.02	100	52.94	0.08	7.69
D19	25,19,0,7	100	26.92	56.82	100	62.75	0.27	26.92
D20	25,13,0,13	100	50.00	65.79	100	74.51	0.50	50.00
D21	25,7,0,19	100	73.08	78.13	100	86.27	0.73	73.08
D22	25,5,0,21	100	80.77	83.33	100	90.20	0.80	80.77
D23	25,2,0,24	100	92.31	92.59	100	96.08	0.92	92.31
				Color Doppler				
D15	25,26,0,0	100	0.00	49.02	NC	49.02	0.00	0.00
D16	25,26,0,0	100	0.00	49.02	NC	49.02	0.00	0.00
D17	24,25,1,1	96	3.85	48.98	50	49.02	−0.01	−0.15
D18	25,23,0,3	100	11.54	52.08	100	54.90	0.11	11.54
D19	25,15,0,11	100	42.31	62.50	100	70.59	0.42	42.31
D20	25,7,0,19	100	73.08	78.13	100	86.27	0.73	73.08
D21	25,2,0,24	100	92.31	92.59	100	96.08	0.92	92.31
D22	25,2,0,24	100	92.31	92.59	100	96.08	0.92	92.31
D23	25,2,0,24	100	92.31	92.59	100	96.08	0.92	92.31

TP, True positive; *FP*, False positive; *FN*, False negative; *TN*, True negative; *Sens*, Sensitivity; *Spec*, Specificity; *PPV*, Positive predictive value; *NPV*, Negative predictive value; *Acc*, Accuracy; *κ*, Kappa Index; *NC*, Not Calculable.

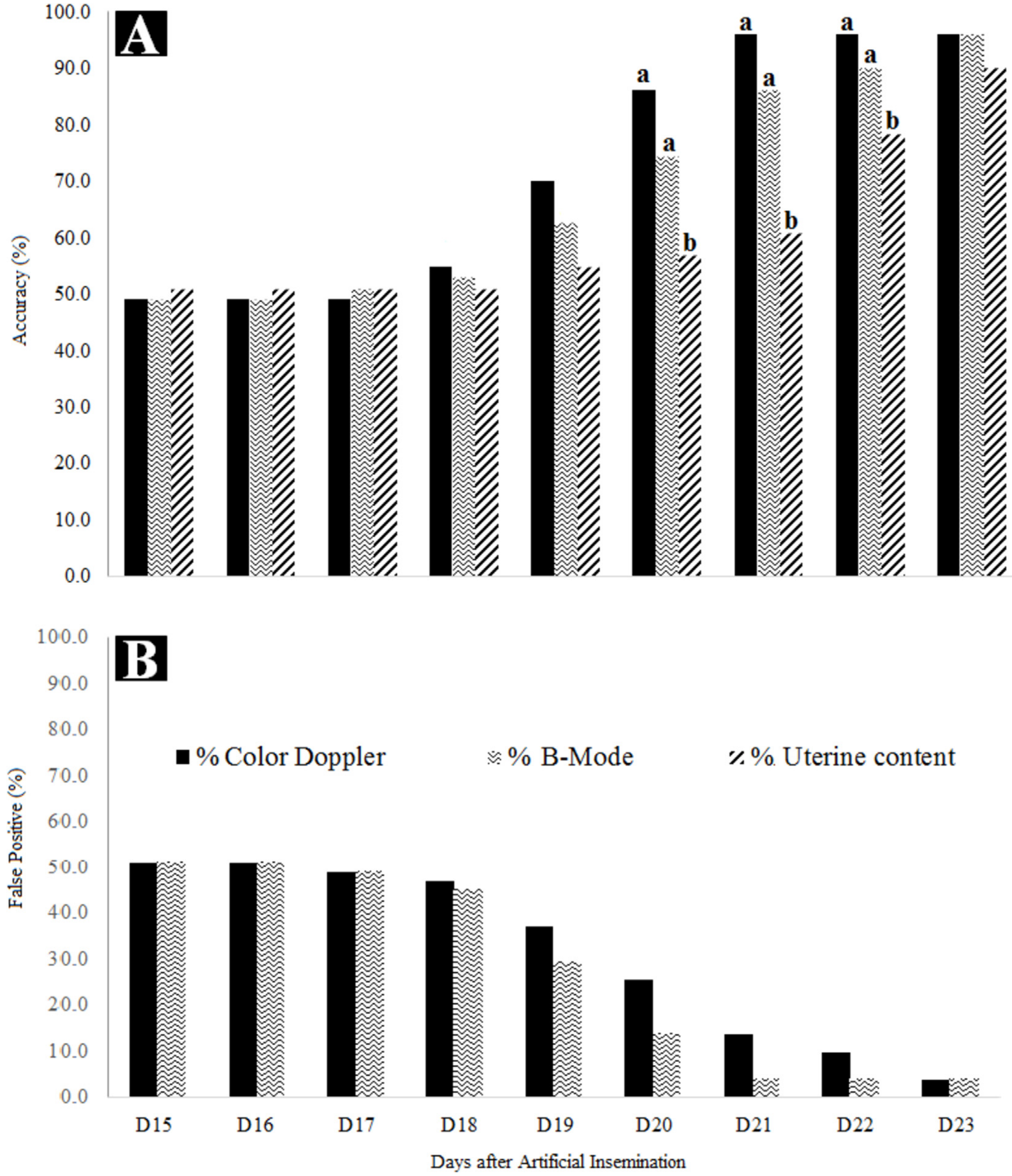

Fig. 3. Color Doppler, B-Mode, and overall assessment agreement on accuracy (A) and color Doppler and B-Mode agreement on False positives (B) in dairy goats (n = 51) from Days 15–23 (estrous cycle) in Study 1.
[a, b] Different letters, into the same day among columns, means a significant difference by Fisher Exact Test (P < 0.05).
* Columns without superscript letters refer to non-significant differences between evaluations by Fisher Exact Test (P < 0.05).
** There was no difference between evaluations compared at part B of this figure by Fisher Exact Test (P < 0.05).

3.2. Study 2

Outcomes of the US assessments on Day 21 by color Doppler (luteal blood-flow assessment), and Day 23 by B-Mode (luteal morphology assessment and overall assessment), compared with Day 30 (pregnancy diagnosis), are shown in Table 3. The evaluations at Day 21 and Day 23 presented a similar pattern of sensitivity and specificity when compared to Study 1.

4. Discussion

In domestic ruminants, pregnancy diagnosis using tranrectal B-Mode US is usually performed around Day 30 post-breeding and is mainly based on a visualization of a viable embryo vesicle with the presence of a heartbeat [1–3]. Our findings demonstrated that pregnancy diagnosis in goats could be accurately performed as early as on Day 21 and Day 23 post-breeding, using luteal assessment by color Doppler and B-Mode US, respectively. Equally, our findings were similar to previous results reported in bovine [4,5,20] and sheep [8,10] species. This possibility of performing an accurate and early pregnancy diagnosis using B-Mode US is probably due to improvements in ultrasound technology and its availability for use in farm animals, such as high-frequency transducers and a higher quality of images.

As expected, the distinction between pregnant and non-pregnant goats by luteal morphology and blood-flow assessment using B-Mode and color Doppler US was not possible at Day 15 and Day 16 post-breeding, as all the CL were considered active and,

Table 2
The uterine content echogenicity assessment (presence of anechoic content) and uterine content echogenicity assessment associated to luteal evaluation for early pregnancy diagnosis in dairy goats (n = 51) from Days 15–23 (post-insemination) in Study 1.

Day of the cycle	TP, FP, FN, TN (n)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Acc (%)	κ	Youden's index
				Anechoic Uterine Content				
D15	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D16	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D17	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D18	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D19	2,2,23,24	8	92.31	50	51.06	50.98	0.01	0.31
D20	3,1,22,25	12	96.15	75	53.19	54.90	0.08	8.15
D21	3,2,22,24	12	92.31	60	52.17	52.94	0.04	4.31
D22	14,0,11,26	56	100	100	70.27	78.43	0.56	56.00
D23	20,0,5,26	80	100	100	83.87	90.20	0.80	80.00
				Uterine Content with Luteal Evaluation[a]				
D15	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D16	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D17	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D18	0,0,25,26	0	100	NC	50.98	50.98	0.00	0.00
D19	2,0,23,26	8	100	100	53.06	54.90	0.08	8.00
D20	3,0,22,26	12	100	100	54.17	56.86	0.12	12.00
D21	5,0,20,26	20	100	100	56.52	60.78	0.20	20.00
D22	14,0,11,26	56	100	100	70.27	78.43	0.56	56.00
D23	20,0,5,26	80	100	100	83.87	90.20	0.80	80.00

TP, True positive; *FP*, False positive; *FN*, False negative; *TN*, True negative; *Sens*, Sensitivity; *Spec*, Specificity; *PPV*, Positive predictive value; *NPV*, Negative predictive value; *Acc*, Accuracy; *κ*, Kappa Index; *NC*, Not Calculable.

[a] Since there was no difference between luteal morphology + uterine content and luteal blood-flow + uterine content, they are shown as a single evaluation at the present table.

Table 3
The overall performance of the luteal and uterine assessment by color Doppler and B-Mode for early pregnancy diagnosis in dairy goats (n = 71) on Days 21 and 23 (post-insemination), respectively, in Study 2.

Type of Assessment	TP, FP, FN, TN (n)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Acc (%)	κ	Youden's index
Doppler (D21)	30,3,0,38	100	92.68	90.91	100	95.77	0.91	92.68
B-Mode (D23)	30,2,0,39	100	95.12	93.75	100	97.18	0.94	95.12
Uterine Content (D23)	30,1,0,40	100	97.56	96.77	100	98.59	0.97	97.56

TP, True positive; *FP*, False positive; *FN*, False negative; *TN*, True negative; *Sens*, Sensitivity; *Spec*, Specificity; *PPV*, Positive predictive value; *NPV*, Negative predictive value; *Acc*, Accuracy; *κ*, Kappa Index.

therefore, non-pregnant does were wrongly classified (FP). Previous studies in goats demonstrated that the luteolysis phase begins, on average, from Day 17 of the estrous cycle [6,9]. Therefore, ultrasonographic changes in luteal morphology and blood flow could only be detectable from this day onward. In this sense, it was seen that the overall performance of the pregnancy diagnosis prediction by B-Mode and color Doppler US improves from Day 17 post-breeding on. Equally, non-pregnant does could be correctly identified (TN) from Day 17 to Day 18 post-breeding using both US techniques. As soon as the luteolysis began to happen, a decrease of the luteal blood flow and morphology scores were detected by the US. In addition, the accuracy has improved because the FP decrease and TN increase. Nevertheless, maximum performance was only reached on Day 21 for color Doppler and Day 23 for B-Mode US, when FP results reached their minimum value and all others parameters reached their maximum values. Similar results were also observed in the second study when these techniques were evaluated in a greater number of does using the days fixed by the first study.

The reason why the luteal blood-flow assessment reached maximum accuracy (D21) two days before the luteal morphology assessment (D23) can be explained by the physiological events sequence that occurred throughout the luteolysis phase (reviewed by Refs. [21–23]). The luteolysis process has two distinct steps, which are functional and structural luteolysis [4,6–9]. In the first step (24 h–48 h after luteolysis onset), a decrease in plasma P4 concentration is observed accompanied by a decrease in luteal blood flow [8,9]. Thereafter (48–72 h after luteolysis onset), significant changes in luteal morphology are observed [7]. As demonstrated by previous studies [9,24], the P4 production is conditioned to a good blood supply (steroid precursors), as well as the P4 release into the blood circulation. Therefore, it is expected that a pregnant doe should have a well-vascularized CL for pregnancy maintenance. Also, during luteolysis in non-pregnant goats, structural changes occur due to the luteal cells' death, which can be represented in the US assessment as the loss of luteal area and volume [9,24]. This process does not happen in the pregnant ones preserving the luteal morphology since it is positively correlated with luteal functionality [5,6,11].

At the end of the first and second study, a final accuracy of between 95 and 97% was obtained to reach the early pregnancy diagnosis by luteal assessment, regardless of B-Mode or color Doppler US. Additionally, the number of FP does on Day 23, in both studies, could be explained by a prolonged estrous cycle, due to the persistence of an active CL, but in absence of uterine fluid. The goat's estrous cycle has an average of 21 days. However, variations between 8 and 39 days on average have been described (short and long cycles, respectively) [25]. It is also important to highlight that embryonic or fetal loss can happen until Day 55, with frequencies that can vary greatly depending on the production system and the health or nutritional challenge that the flock is facing [26].

Previous studies have shown that the presence of intrauterine content, from Day 18 in goats, could be used as a biological marker for early pregnancy diagnosis [19]. In this respect, the present study adopted the uterine assessment in order to obtain an overall evaluation of the female reproductive tract. Nevertheless, the presence of anechoic uterine content was only observed from Day 19 on ward and, when evaluated alone, reached its maximum accuracy on Day 23. Thus, under experimental conditions, the evaluation of the uterine content echogenicity was not reliable. Likewise, the luteal assessment showed a greater accuracy for early pregnancy prediction, regardless of the presence of anechoic uterine content or not. Moreover, the uterine content echogenicity takes a longer time to reach a TP result when compared to the CL maintenance (lack of luteolysis due to maternal recognition of pregnancy). It was also found the presence of some FP does for the anechoic uterine content from Day 19 to Day 21 which could be justified as a result of a subsequent estrogenic phase from the proestrus or estrus phase in non-pregnant does [19], since the anechoic image in the uterus was not related to a active CL in the same animal.

To our knowledge, this is the first study to propose and compare both techniques for early determination of the pregnancy status in does. Such findings enable an intensification of breeding programs, as non-pregnant does can be resubmitted to a second AI protocol after a shorter period when compared to a classical US pregnancy diagnosis (on Day 30 post-breeding). Moreover, the proposed methodology may be a very useful alternative for reproductive management during non-breeding season, as pregnancy diagnosis based on signs of estrus return cannot be reliably performed [27]. The earliest pregnancy diagnosis can also be adopted in resynchronization programs, in order to avoid a new ovulation and/or the necessity of additional hormones in the protocol, such as $PGF_{2\alpha}$, which could result in a loss of an undetected pregnancy. Also, for those programs, other gonadotrophins could be used, like hCG or pFSH, in order to avoid eCG-antibodies in the goat species [28]. However, when the aim is just an early pregnancy diagnosis for selling or flock management, it can be performed on Day 23 for a most accurate exam, even in view of the possible embryonic losses in the sequence [26].

5. Conclusions

A subjective assessment of the luteal blood flow by color Doppler US and luteal morphology by B-Mode US is an effective tool for the early pregnancy diagnosis in goats on Day 21 and 23 post-breeding, respectively. This approach is made more crucial by the fact that the assessment of uterine content echogenicity, in association with a functional CL, was not sufficiently accurate to reach an earlier pregnancy diagnosis when compared to the luteal assessment.

Authors' contributions

FZB, MFAB, IOC conceived the project and coordinated the study. All authors contributed to the experimental activities. FZB, MFAB, IOC and EKNA drafted the manuscript, which was revised by all authors. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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