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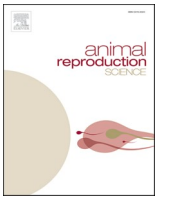

Effect of a 12-h increment in the short-term treatment regimen on ovarian status, estrus synchrony, and pregnancy rate in artificially inseminated dairy goats

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ABSTRACT

This study was conducted to assess effects of two hormonal treatments on ovarian follicular status, estrous synchrony and fertility in dairy goats during the non-breeding season when duration of progestogen device use varied by 12 h. In both experiments, does were administered 60 mg of medroxyprogesterone acetate via intravaginal devices, respectively, for 6 and 6.5 d (G6 and G6.5). At 24 or 36 h before device removal, 200 IU of eCG im and 30 µg d-cloprostenol im were administered. In Experiment 1 (n = 24), data related to sexual behavior and that were collected using ovarian ultrasonography were recorded, and in Experiment 2 (n = 83) fertility was assessed after Flexible Time Artificial Insemination (FxTAI). The interval from device removal to estrus was shorter ($P < 0.05$) after imposing the G6.5 treatment regimen. Diameter of largest and second-largest ovarian follicles and interval from device removal to ovulation were similar ($P > 0.05$) between groups. The does treated with the G6.5 hormonal regimen had greater estrous synchrony, associated with greater development of largest follicles at the time of device removal, which might have led to a lesser fertility rate ($P > 0.05$). Conversely, treatment with the G6 hormonal regimen resulted in a greater conception rate. In conclusion, increasing time the intravaginal device is inserted from 6 to 6.5 d resulted in greater estrous synchrony, advanced ovarian follicular development, abnormal CL function and lesser pregnancy rates in artificially inseminated dairy goats when there were treatments during the non-breeding season.

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1. Introduction

In southeast Brazil, as well as in other countries, goats have marked reproductive seasonality, with a distinct seasonal anestrous period in the spring (Balaro et al., 2019). Using reproductive techniques to overcome this circumstance, therefore, is important for improving reproductive efficiency in dairy goats. Certain strategies are adopted to avoid variation in milk production throughout the year, such as the induction of synchronized timing of estrus among does with use of hormonal treatment regimens. There is great variation among studies when there is use of these treatment regimens, such as in the use of different hormonal doses (Gómez et al., 2006), times of administration, sites of administration, and duration in times the progestogen device is located intravaginally (Nascimento-Penido et al., 2018). In general, the use of a progestogen device results in a relatively shorter-, medium-, or longer-period of sustained estrous cycles in a large proportion of the anestrous does responding to these treatments (Pietroski et al., 2013). Notably, treatment regimens of a shorter interval result in increased responses in naturally mated ewes (Viñoles et al., 2001) and goats (Fonseca et al., 2005b).

The variation in the interval between onset of behavioral estrus and intravaginal device removal among does continues to be the main issue adversely affecting fertility after fixed-time artificial insemination (FTAI) in goats. This is why Artificial Insemination (AI) has been performed based on time of estrous onset (i.e., Flexible Time AI – FxTAI) in estrous cyclic dairy goats treated with two doses of d-cloprostenol, with there being conception rates of 85% to 94%) (Maia et al., 2017; Bonato et al., 2019). Nevertheless, the use of FxTAI after synchronous estrous induction with use of progestogen treatment regimens has not yet been assessed in dairy goats managed in tropical conditions during the non-breeding season. Anestrous goats treated with short-term hormonal regimens for synchronous estrous induction (6 d progestagen combined with cloprostenol and eCG) and subjected to FTAI (≅ 54 h after device removal) had pregnancy rates ranging from 46% to 64% (Fonseca et al., 2019).

The onset of estrus often occurs during the night hours when there is use of many of the hormonal treatment regimens for induction of estrus in anestrous does (Fonseca et al., 2005a). Maia et al. (2017) proposed the 11.5-d interval to allow for the administration of the second d-cloprostenol, in the late afternoon. The reason for the large incidence of estrous onset during the night hours is likely associated with the dominant ovarian follicular status at the time of the second cloprostenol administration. Most estrous cyclic dairy goats treated with two doses of d-cloprostenol at a 7.5 d interval expressed the initial symptoms of behavioral estrus during the hours when there was daylight on the second day after the second cloprostenol administration, with there being a tendency towards a greater proportion of goats being pregnant when there was imposing of this treatment regimen before the breeding of does (Bonato et al., 2019). It, therefore, was hypothesized that in the non-breeding season, when there was a prolonging time of use of the progestogen device when imposing the standard treatment regimen (insertion and removal of progesterone device at the end of the afternoon; Fonseca et al., 2017a) for 12 h (6 d compared with 6.5 d) could prevent the earlier than desirable estrous onset (i.e., for as long as 24 h after device removal), allowing for a greater number of does to be in estrus at the same time. If this hypothesis is confirmed, the strategy of extending the progestogen for 12 h could further support the greater efficiency of FTAI and should be the subject of future research. The present study, therefore, was conducted to investigate the effects of extending the length of time the progestogen device was intravaginal by 12 d. For subjecting dairy does to FxTAI, this treatment was imposed during the seasonal anestrous period for synchronous estrous induction so as to evaluate estrous response, and the ovulatory and fertility variables.

2. Materials and methods

2.1. Ethics and animal care

This study was approved by the Ethics Committee for the Use of Animals of Universidade Federal Fluminense (protocol #6405230719) and was conducted in ways consistent with the principles of the Brazilian Society of Laboratory Animal Science.

2.2. Location and experimental conditions

The study comprised two experiments, both undertaken during the non-breeding season, from September (animal selection and synchronized estrous induction) to December (pregnancy diagnosis). Experiment 1 was conducted at Embrapa's Experimental Campus in Coronel Pacheco (21° 35' S and 43° 15' W) and Experiment 2 was conducted at a commercial dairy goat farm in Ouro Fino (22° 16' S and 46° 22' W), both in the state of Minas Gerais, Brazil.

All does were maintained in an intensive system, in pens, with there being natural photoperiodic cues and at room temperature, and fed a corn silage diet. A balanced concentrate was provided according to the nutritional requirements of the animals (National Research Council-NRC, 2007). Mineralized salt (Caprinofós® Tortuga, São Paulo, Brazil) and drinking water were available *ad libitum*.

2.3. Experimental animals and treatments

In both experiments, Alpine ($n = 65$) and Saanen ($n = 42$) goats were assessed for clinical health and reproductive status, as well as body condition score (BCS; 1 = very thin and 5 = very fat; Villaquiran et al., 2007). In Experiment 1, goats were equally distributed according to breed, body weight, age, parity order, and BCS into two groups to be treated with intravaginal devices containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Zoetis, São Paulo, Brazil) for 6 (G6; $n = 12$, body weight: 56.8 ± 4.2 kg and BCS: 3.0 ± 0.1) or 6.5 d (G6.5; $n = 12$, body weight: 59.5 ± 3.2 kg and BCS: 3.0 ± 0.1). In does of both groups, devices were inserted at the same time, at the end of the afternoon (1700 to 1800 h), while device removal occurred at the end of the afternoon (G6: 1700 to

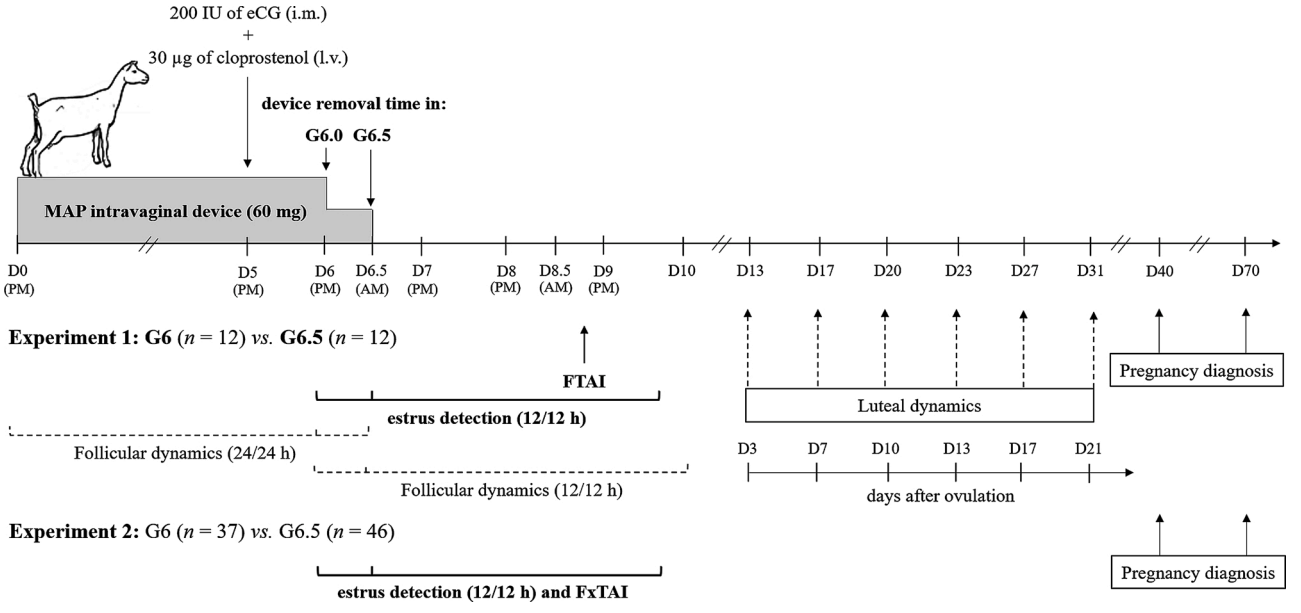

Fig. 1. Schematic representation of the experimental design to evaluate the use of 60 mg medroxyprogesterone acetate (MAP) intravaginal devices for 6 d (G6) or 6.5 d (G6.5) in an estrous induction treatment regimen in dairy goat does during the non-breeding season; eCG: equine chorionic gonadotrophin; FTAI: Fixed time artificial insemination; FxTAI: Flexible time artificial insemination.

1800 h) or the following morning (G6.5: 0500 to 0600 h). In addition, all does were administered 200 IU of eCG (Novormon 5000®, Zoetis, São Paulo, Brazil) im, plus 30 µg d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) im at 24 h (G6) or 36 h (G6.5) before device removal (Fig. 1). In Experiment 2, does assigned to the G6 ($n = 37$, BCS: 3.2 ± 0.1) and G6.5 ($n = 46$, BCS: 3.2 ± 0.1) groups were administered the same treatments as in Experiment 1.

2.4. Ovarian follicular dynamics

Ovarian follicular dynamics were assessed in Experiment 1 every 24 h from the day of device insertion until removal, and every 12 h from device removal until the time ovulation was detected to have occurred, by the same operator. Ultrasonic equipment (Mindray® M5Vet, Shenzhen, China) was used, coupled to a 7.5 MHz linear transducer. The transducer was adapted to a PVC tube cut longitudinally so that it could be manipulated when inserted via the transrectal route. The does were maintained in a quadrupedal position in an enclosure suitable for small ruminants. After feces were removed, 10 mL of carboxymethylcellulose gel was placed in the rectum, using a 60 mL syringe. The transducer was placed in the rectum until the bladder and uterus were visualized, and the visualization of the ovaries occurred as a result of rotating the transducer laterally to both sides (De Castro et al., 1999). The diameter (mm), position, and characteristics of the ovarian structures were recorded for individual does.

Data regarding follicular wave emergence, follicular growth dynamics, and ovulation were recorded (De Castro et al., 1999). The time of ovulation was considered to be when the dominant follicle(s), identified previously, was (were) no longer present at the time of ultrasonic assessment. Ovarian follicular status at the time immediately preceding device removal was classified as "early developmental" when the largest follicles were <6.0 mm diameter and "advanced development" when ≥ 6.0 mm diameter.

2.5. Estrous behavior and artificial insemination

In both experiments, symptoms of behavioral estrus were monitored twice daily after device removal to 96 h after removal, aided by use of "teaser" bucks. Goats were considered to be in estrus when standing to be mounted. The AI was performed using the Embrapa® transcervical technique in goat does by the cervical immobilization technique (Fonseca et al., 2017a) at a time that was determined that was consistent with the time of ovarian follicular emergence so that there were oocytes released at the time of ovulation that were of a similar developmental stage in does of both groups. In Experiment 1, the FTAI, therefore, was performed at the end of the morning (1000 to 1200 h), at 64 to 66 h and 52 to 54 h after device removal in the does of the G6 and G6.5 groups, respectively. In Experiment 2, the AI time was adjusted based on time of detection of symptoms of behavioral estrous onset. The AI was performed as previously described for FxTAI; however, the time of intravaginal device removal was used to determine time of FxTAI instead of the time of the second administration of d-cloprostenol based on results from previous studies (Maia et al., 2017; Bonato et al., 2019). Does initiating estrus earlier, at an intermediate time, or later were inseminated at 24, 18, or 10 h after estrous onset, respectively (Table 1). Semen was donated by CapraGene®, the Brazilian Breeding Plan group for dairy goat progeny testing (Facó et al., 2011). Semen was frozen with 100 to 120 million viable sperm per 0.25 mL straw before freezing and spermatozoa had been previously evaluated and determined to have at least 40% progressive linear motility (0% to 100% variation) and a vigor score of 3 (speed of viable sperm cells; 0 to 5 variation) after thawing in a water bath at 35 °C for 30 s.

2.6. Luteal evaluation and pregnancy

The corpora lutea (CL) evaluations were performed in Experiment 1 on Days 3, 7, 10, 13, 17, and 21 after the day of detection that ovulation had occurred, using the B-mode and color Doppler mode ultrasonic device. The Doppler settings used in the luteal assessments were the same throughout the experiment, as follows: 75% color gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth, and wall filter (WF) of 75 MHz. After locating each ovary, the number of CL were quantified with each image analysis occurring at a later time in a darkroom (Figueira et al., 2015). Diagnosis for pregnancy status was assessed in all does at 60 d after AI.

2.7. Variables and statistical analysis

The following values for the variables were determined: Follicular wave emergence after intravaginal device insertion or removal

Table 1
Regimen for Flexible Time Artificial Insemination (FxTAI) based on estrous onset in dairy goat does on which there was imposing of estrous induction treatments* during the non-breeding season

	Treatment	
Estrous onset after device removal	6 d	6.5 d
24 h	24 h later (late afternoon)	24 h later (early morning)
36 h	24 h later (early morning)	18 h later (late morning)
48 h	18 h later (late morning)	10 h later (late afternoon)
60 h	10 h later (late afternoon)	-

* Estrous induction treatments: 60mg of medroxyprogesterone acetate intravaginal devices for 6 or 6.5 d; Both 200 IU of eCG im and 37.5 µg of d-cloprostenol im were administrated at 24 h (G6) or 36 h (G6.5) before intravaginal device removal.

(the day a follicle developed to 3 mm in diameter followed by an increase to 4 mm the subsequent day) (h); diameter of the largest follicle on the day of intravaginal device removal (mm); diameter of the second largest follicle on the day of intravaginal device removal (mm) (De Castro et al., 1999); estrous response rate (number of does in estrus/number of does on which estrous synchrony regimens were imposed × 100); interval from intravaginal device insertion or removal to estrous onset (h); interval from intravaginal device insertion and removal to ovulation (h); interval from follicular wave emergence from intravaginal device insertion or removal (h); diameter of the largest and second largest ovulatory follicle (mm); average diameter of follicles from which there were ovulations (mm); follicular growth rate per day (mm/d); interval from follicular wave emergence to time when ovulation was detected to have occurred (h); percentage of does having ovulations (number of does with confirmed ovulation/number of does evaluated using ultrasonography × 100); ovulations (total number of ovulations/number of does having ovulations × 100); CL on Day 7 to 21 after estrous onset (*n*); interval from intravaginal device removal to FxTAI (h); interval from estrous onset to FxTAI (h); conception rate (number of pregnant does/number of does artificially inseminated × 100); pregnancy rate (pregnant does/number of does on which the estrous induction treatment regimen was imposed); and goats with functional CL (animals functional CL/total number of does having ovulations × 100).

There was considered to be a mean difference when there was a $P < 0.05$. Lilliefors and Bartlett tests were conducted to determine if there were normality and homoscedasticity of data for variables, respectively. Parametric variables were subjected to one-way ANOVA by the SAEG program. Nonparametric variables were analyzed using the Chi-square test or Fisher exact test. The results are described as mean ± S.E.M, and categorical results are presented as percentages.

3. Results

The values for reproductive variables Experiment 1 are reported in Table 2. Data related to follicular profile at time of device removal in Experiment 1 are included in Table 3. There was an "advanced development" follicular status (with the largest follicles ranging from 6.2 to 8.2 mm in diameter) in 54.2% (13/24) of does, being 30.7% (4/13) and 69.3% (9/13) for the G6 and G6.5 groups, respectively ($P = 0.0576$). There was the "early developmental" follicular status (with the largest follicles ranging from 4.8 to 6.0 mm in diameter) in 45.8% (11/24) of does, being 63.6% (7/11) and 36.4% (4/11) from G6 and G6.5 d groups, respectively ($P = 0.197$). One doe with an "early developmental" and three does with an "advanced development" follicular status did not have an ovulation and data from these does were not included in Table 3. There were two and four pregnant does of the G6 and G6.5 groups that had an "advanced development" follicular status at the time these assessments occurred during the estrous synchrony treatment regimen. Data for presence of functional CL from Day 3 to 21 after ovulation are depicted in Fig. 2.

Data for reproductive variables in Experiment 2 are reported in Table 4. Considering both experiments, the does of the G6 and G6.5 groups had overall estrous responses of 83.7% (41/49) and 89.7% (52/58), respectively ($P > 0.05$), while the overall pregnancy rate was greater ($P < 0.05$) in does of the G6 (63.3% or 31/49) than G6.5 (43.1% or 25/58) group.

Table 2
Data for reproductive variables of dairy goats submitted to fixed time artificial insemination (FTAI) after imposing an estrous induction treatment regimen* during the non-breeding season (Experiment 1)

	Treatments		
End points	6 d	6.5 d	*P* value
Animals (*n*)	12	12	
Follicular wave emergence time after device insertion (h)	76.3 ± 5.4	74.0 ± 5.4	n.s.
Follicular wave emergence time after device removal (h)	68.4 ± 3.9	71.3 ± 5.2	n.s.
Diameter of the largest follicle at device removal (mm)	5.9 ± 0.3	6.5 ± 0.3	n.s.
Diameter of the second largest follicle at device removal (mm)	5.0 ± 0.3	5.4 ± 0.2	n.s.
Estrous response (%)	91.7	100.0	n.s.
Interval from device insertion to estrous onset (h)	188.4 ± 3.3	190.7 ± 1.8	n.s.
Interval from device removal to estrous onset (h)	44.4 ± 3.3	34.7 ± 1.8	0.009
Interval from device insertion to ovulation (h)	219.9 ± 3.6	228.8 ± 5.3	n.s.
Interval from follicular wave emergence to ovulation (h)	112.1 ± 6.8	117.7 ± 5.3	n.s.
Interval from device removal to ovulation (h)	76.0 ± 0.4	72.0 ± 0.5	n.s.
Interval from estrus onset to ovulation (h)	31.5 ± 4.0	37.1 ± 5.3	n.s.
Diameter of the largest ovulatory follicle (mm)	8.3 ± 3.6	8.2 ± 4.2	n.s.
Diameter of the second largest ovulatory follicle (mm)	6.7 ± 3.4	7.4 ± 3.2	n.s.
Average diameter of the ovulatory follicles (mm)	7.9 ± 4.0	7.7 ± 3.4	n.s.
Follicular growth rate per day (mm/d)	0.8 ± 0.1	0.8 ± 0.1	n.s.
Animals ovulating (%)	91.7	100.0	n.s.
Ovulations (*n*)	1.8 ± 0.2	1.7 ± 0.2	n.s.
Corpora lutea at Day 7 after estrous onset (n)	1.8 ± 0.2	1.8 ± 0.2	n.s.
Interval from device removal to FTAI (h)	65.9 ± 0.5	52.6 ± 0.2	0.001
Interval from estrous onset to FTAI (h)	21.5 ± 3.3	17.9 ± 1.7	n.s.
Conception (%)	54.5 (6/11)	33.3 (4/12)	n.s.
Pregnancy (%)	50.0 (6/12)	33.3 (4/12)	n.s.

* Estrous induction treatments: 60 mg of medroxyprogesterone acetate intravaginal devices for 6 or 6.5 d. Both 200 IU of eCG im and 37.5 μg of d-cloprostenol im were administrated at 24 h (G6) or 36 h (G6.5) before intravaginal device removal.

Table 3
Data for reproductive variables based on the follicular status observed at the time of intravaginal device removal in dairy goat does submitted to fixed time artificial insemination (FTAI) after estrous induction treatment regimen* during the non-breeding season (Experiment 1)

	Ovarian follicular status		
End points	Early Developmental**	Advanced development #	*P* value
Animals (*n*)	11	13	
Follicular wave emergence after device insertion (h)	82.9 ± 6.7	68.0 ± 2.7	0.04
Follicular wave emergence after device removal (h)	78.0 ± 4.7	62.6 ± 3.3	0.01
Diameter of the largest follicle at device removal (mm)	5.4 ± 0.1	6.9 ± 0.2	0.001
Diameter of the second largest at device removal (mm)	4.6 ± 0.1	5.7 ± 0.2	0.001
Estrous response (%)	100.0	92.3	n.s.
Interval from device removal to estrous onset (h)	43.8 ± 2.5	35.9 ± 2.9	0.08
Interval from device removal to ovulation (h)	81.1 ± 4.8	67.1 ± 3.4	0.04
Interval from estrous onset to ovulation (h)	38.0 ± 5.7	31.1 ± 3.7	n.s.
Interval from follicular emergence to ovulation (h)	108.9 ± 7.0	120.6 ± 4.6	n.s.
Diameter of the largest ovulatory follicle (mm)	8.3 ± 0.4	8.2 ± 0.4	n.s
Diameter of the second largest ovulatory follicle (mm)	6.9 ± 0.4	7.3 ± 0.3	n.s
Average diameter of the ovulatory follicles (mm)	7.9 ± 0.4	7.7 ± 0.3	n.s
Follicular growth rate per day (mm/d)	0.9 ± 0.1	0.6 ± 0.1	0.06
Ovulations (n)	1.8 ± 0.2	1.7 ± 0.2	n.s
Corpora lutea at Day 7 after estrous onset (n)	1.9 ± 0.2	1.7 ± 0.2	n.s
Interval from device removal to FTAI (h)	60.9 ± 2.0	57.2 ± 2.0	n.s
Interval from estrous onset to FTAI (h)	17.8 ± 2.4	21.3 ± 2.7	n.s
Conception (%)	36.6 (4/11)	50.0 (6/12)	n.s
Pregnancy (%)	36.6 (4/11)	46.2 (6/13)	n.s

* Estrous induction treatments: 60 mg of medroxyprogesterone acetate intravaginal devices for 6 or 6.5 d. Both 200 IU of eCG im and 37.5 μg of d-cloprostenol im were administrated at 24 h (G6) or 36 h (G6.5) before intravaginal device removal.
** Follicle < 6.0 mm diameter.
# Follicles ≥ 6.0 mm diameter.

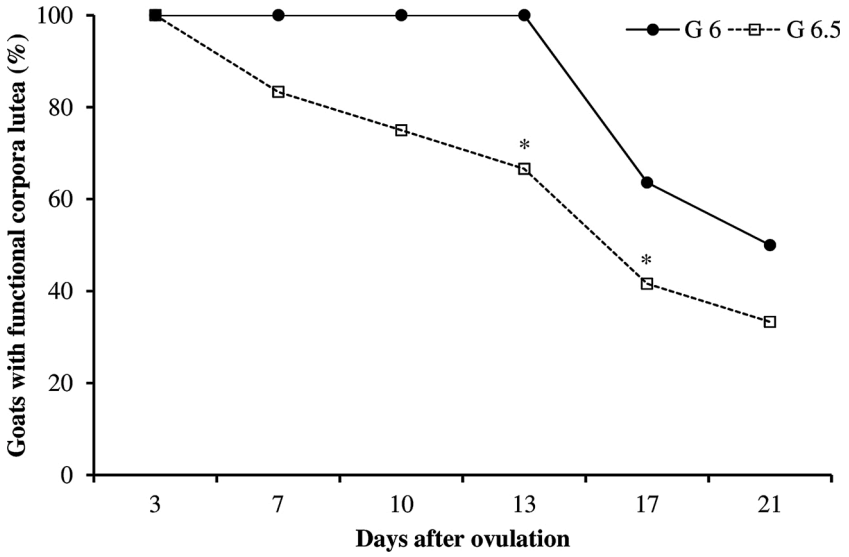

Fig. 2. Percentage of dairy goat does with functional corpora lutea from Day 3 to 21 after ovulation (Experiment 1); Does were previously subjected to Flexible Time Artificial Insemination (FxTAI) after imposing an estrous induction treatment regimen with 60 mg of medroxyprogesterone intravaginal devices for 6 or 6.5 d combined with 200 IU of eCG im and 37.5 μg of d-cloprostenol im both administrated at 24 or 36 h before intravaginal device removal, respectively, during the non-breeding season; *Two does of the G6.5 group had partial luteal regression between Days 10 and 13 and Days 13 and 17 and were pregnant at the time of pregnancy status assessments.

4. Discussion

Both treatments were effective in inducing a synchronized estrous response during the non-breeding season in dairy goats, when there were conditions similar to those reported by Fonseca et al. (2005a, b). Notably, the interval from intravaginal device removal to estrous onset was less for does of the G6.5 (34.7 h), while that for G6 (44.4 h) group was similar to those described previously (Fonseca et al., 2005a, b; Fonseca et al., 2017b). Prolonging by 12 h the time the intravaginal device was in place was directly reflected in the

Table 4
Data for reproductive variables of dairy goat does submitted to flexible time artificial insemination (FxTAI) after imposing an estrous induction treatment regimen* during the non-breeding season (Experiment 2)

Variables	6 d	6.5 d	*P value*
Animals (*n*)	37	46	-
Estrous response (%)	81.0 (30/37)	86.9 (40/46)	n.s
Interval from device removal to estrous onset (h)	46.8 ± 2.1	38.1 ± 1.4	0.04
Interval from device insertion to FxTAI (h)	213.1 ± 2.5	213.0 ± 1.4	n.s.
Interval from device removal to FxTAI (h)	67.3 ± 1.9	56.1 ± 1.1	0.001
Interval from estrus onset to FxTAI (h)	20.8 ± 1.5	18.1 ± 1.4	n.s.
Conception (%)			
Estrous onset 24 h after device removal	100.0 (4/4)	64.3 (9/14)	n.s.
Estrous onset 36 h after device removal	88.9 (8/9)	50.0 (2/4)	n.s.
Estrous onset 48 h after device removal	60.0 (3/5)	45.4 (10/22)	n.s.
Estrous onset 60 h after device removal	83.3 (10/12)	-	-
Overall	83.3 (25/30)	52.5 (21/40)	0.01
Pregnancy (%)	67.6 (25/37)	45.6 (21/46)	0.05

* Estrous induction treatments: 60 mg of medroxyprogesterone acetate intravaginal devices for 6 or 6.5 d. Both 200 IU of eCG im and 37.5 μg of d-cloprostenol im were administrated at 24 h (G6) or 36 h (G6.5) before intravaginal device removal.

time of estrus, with does of the G6.5-group initiating symptoms of behavioral estrus approximately 10 h earlier after the device was removed. Interestingly, both the interval from device insertion to estrous onset and from follicular wave emergence to ovulation were similar between does of the two groups. Even though there was a shorter interval to estrus, the ovulatory follicles and oocytes released at the time of ovulation probably were of a similar developmental stage when there was the same relative wave of follicular development in does of the two groups. In addition, as hypothesized for the present study, prolonging the length of time the progestogen device was in place intravaginally by 12 h resulted in an excellent estrous response (~90%) and greater synchrony in timing of estrus among does (initiating estrus about 24 h after device removal). The use of the G6.5 hormonal treatment regimen could result in a reduction in the time required for estrous monitoring when using FxTAI.

The diameter of the largest dominant follicle and follicular growth rate were similar for does when there was imposing of the G6 and G6.5 treatment regimens (~8.25 mm and 0.8 mm/d), corroborating data previously reported (De Castro et al., 1999). There, however, were interesting data in the present study related to the ovarian follicular status at the time of intravaginal device removal that were quite similar to those for sheep (Menchaca and Rubianes, 2004). The "advanced development" follicular status associated with shorter period to estrous onset after intravaginal device removal tended to be more frequent in the does of the G6.5 (69.3%) than in the G6 (30.7%) group and follicular growth rate tended be less in "advanced development" (0.6 mm/d) than for "early developmental" (0.9 mm/d) status follicles. After subjecting ewes to short- (6 d) or long- (12 d) term treatment regimens, Viñoles et al. (2001) reported that there were differences in stage of dominant follicle development with there being advantages to using the short-term treatment regimen. The prolonged lifespan of dominant follicles with use of the long- compared with the short-term treatment regimen was assumed to be the cause of the lesser pregnancy rate when there was use of the long-term treatment regimen. In the present study, there was assessment of the dominant follicle variables when these follicles had developed during the same wave of follicular dynamics during the hormonal treatment regimens that were imposed. The additional 12 h of device presence intravaginally tended to be associated with the "advanced development" status of dominant follicles, which could be associated with certain conditions affecting follicular growth and, consequently, follicular viability. Furthermore, the more advanced follicular development at the time of intravaginal device removal could have adverse effects on oocyte viability and characteristics of the CL that develops after ovulation from these follicles.

The CL were observed on Day 3 after ovulation, using color Doppler evaluation to characterize CL viability, as proposed by Figueira et al. (2015). Partial and total CL regression was identified in does from both groups in the present study. While precocious and intermediate CL regression was observed in does of the G6.5 group, CL regression was observed only at the expected time of physiological luteolysis in does of the G6-group, after Day 13 of the estrous cycle (Arashiro et al., 2010). Partial CL regression, as previously reported in goat does (Souza-Fabjan et al., 2013) and ewes (Viñoles et al., 2004), was observed from Day 17 to 21 only in does of the G6.5-group, however, this CL response was not associated with the pregnancy status of does in the present study. Considered together, these data indicate that prolonging the time the progestogen device is used could adversely affect dominant follicle viability and CL formation, which could be reflected as inadequate uterine milieu conditions for embryo development and pregnancy recognition as well as subsequent maintenance of pregnancy.

Based on the results from Experiment 1 for the interval from follicular wave emergence to ovulation, FxTAI time (213 h after device insertion) was adjusted in Experiment 2 to allow for spermatozoa and oocytes to be of an optimal developmental stage for fertilization to occur in the female reproductive tract. Even so, conception rates were greater when all estrous onset times were evaluated, culminating in a greater proportional conception rate in does of the G6 (83.3%) than G6.5 (52.5%) group. Although a similar FxTAI strategy was imposed for does of both groups, there could have been inadequate conditions imposed on does of the G6.5 group that could affect gamete and/or CL viability as indicated by results from Experiment 1 and by the lesser conception rates in Experiment 2.

The G6 treatment regimen was previously used in association with FTAI at 51 to 54 h after intravaginal device removal, resulting in a 62.5% pregnancy rate (Fonseca et al., 2017a). In Experiment 2 of the present study, only does in estrus were inseminated and the AI time was based on time of estrous onset. This adjustment probably contributed to the favorable conception rate of 83.3% in does of this

treatment group. The pregnancy rate in both experiments (including animals not detected in estrus) was less being 63.3%, similar to that reported when there was FTAI in the study of Fonseca et al. (2017a) in which there was a similar treatment regimen imposed with sponges inserted and removed early in the morning. In addition, the ease of characterizing estrus in the does and additional potential increase in conception rates when using an FxTAI strategy based on estrous onset, it does not appear reasonable to apply FTAI to dairy goats in tropical conditions. It should be highlighted that FTAI in these conditions implies less time taken for estrous detection, however, more when the AI technique is used and semen is processed.

5. Conclusion

The increase of the time a device is in place intravaginally from 6 to 6.5 d resulted in greater estrous synchrony, but lesser fertility in dairy goats subjected to estrous induction treatment during the non-breeding season. Prolonging the period for the hormonal treatment regimen led to an advanced development of the follicular status after intravaginal device removal, affecting the ovulatory follicle and CL, culminating in lesser conception rates after FxTAI. The G6 treatment regimen is recommended for FxTAI in dairy goats when these goats are located in tropical conditions.

Author Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors contributions

JFF and JMGS-F elaborated the hypothesis and proposed the experimental design. CJCP collected the data from the animals, analyzed the data and wrote the first version of the manuscript. JDG and JHD assisted with the animals and helped collecting and analyzing the data. JFF, MEFO and JMGS-F discussed the design of the experiment and analyzed the data. GNS elaborated and worked on the statistics. CJCP, JFF, MEFO and JMGSF approved the final version of the manuscript.

Declaration of Competing Interest

All authors declare that they do not have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

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