




Use of two cloprostenol administrations 11.5 days apart efficiently synchronizes oestrus in photostimulated multiparous dairy goats in the non-breeding season

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Abstract

This study assessed the efficiency of synchronous oestrous induction by light programme followed by two doses of cloprostenol in acyclic Saanen goats of different parity orders. Primiparous ($n = 22$) and multiparous ($n = 33$) goats were subjected to 16 hr of light and 8 hr of darkness for 60 days (D0-D60), starting 10 days after the winter solstice. All goats received 120 µg cloprostenol doses on D130 (morning) and D141.5 (afternoon) (11.5 days apart). Oestrus behaviour, ovarian follicular dynamics and serum progesterone (P4) analyses were recorded from D0 to D174 at different intervals. Animals in oestrus after D141.5 were randomly assigned into two groups: assisted natural mating (NM) or artificial insemination (AI; 10–24 hr after oestrus onset with frozen-thawed semen). From D57 to D120, 89.0% of goats presented large follicles (5–8 mm) and P4 concentrations were subluteal from D0 to D120. More multiparous compared to primiparous goats (54.5%, 18/33 vs. 18.2%, 4/22) exhibited oestrus after both injections. More primiparous compared to multiparous goats (54.5%, 12/22 vs. 12.1%, 4/33) did not exhibit oestrus after any injection. A total of 35 goats (64%) were in oestrus after the second prostaglandin injection and were subjected to NM or AI. The conception rate was similar among primiparous (70.0%, 7/10) and multiparous (68.0%, 17/25) goats but the pregnancy rate differed, being 31.8% (7/22) and 51.5% (17/33), respectively. No interaction was found between parity order and P4 concentrations in does that became pregnant or not. Thus, the association between light programme (60 days, starting at the beginning of winter) and two cloprostenol administrations 11.5 days apart (starting 70 days after the end of the light treatment) resulted in sufficient synchronous oestrous response in multiparous acyclic Saanen goats to reach satisfactory fertility levels after both NM and AI.

KEYWORDS

anestrus, artificial insemination, corpus luteum, prostaglandin, ultrasonography

1 | INTRODUCTION

Goat milk production in developing countries is of paramount importance. To achieve profitable milk production, animals from European breeds have been imported, such as Alpine, Nubian, Saanen and Toggenburg goats. Although the Saanen breed has great value due to its high milk yield, these animals show a high degree of seasonality, presenting anestrus in the spring (Balara et al., 2019). Due to this physiological restriction, the supply of dairy goat products to the market and end consumers is inconstant, impairing the production chain (Chemineau, Malpau, Brillard, & Fostier, 2007). Therefore, it is essential that oestrous induction tools are applied by dairy goat farmers in the non-breeding season.

Hormonal-based protocols, including medroxyprogesterone acetate (MAP) intravaginal sponges and equine chorionic gonadotropin (eCG), are commonly used to induce synchronous oestrus resulting in an adequate pregnancy rate after natural mating (NM) (Fonseca et al., 2008) or artificial insemination (AI) (Fonseca et al., 2017a) in Brazilian dairy farms showing reproductive seasonality. Despite the reported efficiency of eCG, it is well-known that its continuous use has an impact on immune response (Baril, Remy, Leboeuf, Beckers, & Saumande, 1996). eCG production entails the collection of large amounts of blood and, in certain countries, pregnancies have been lost in mares involved in eCG production (Vilanova, Briyne, Beaver, & Turner, 2019). Likewise, as it is recommended that milk is discarded after 60 days, the use of MAP sponges has also been restricted. Because melatonin implants are not allowed in Brazil, the other alternative is to artificially invert the annual cycle with long-day and short-day regimes. The artificial photoperiod programme (light programme) induces oestrus during the non-breeding season by complex neuroendocrine pathways (Chemineau, Bodin, Migaud, Thiéry, & Malpau, 2010).

Artificial photoperiod treatments are considered a natural, non-invasive and relatively low-cost method of oestrous induction during the non-breeding season; moreover, they respect animal welfare considerations and do not leave residues in milk (Chemineau et al., 2007). However, the oestrus induced during anestrus time (Flores et al., 2013) is not synchronized, as reported by using hormonal protocols (Fonseca et al., 2017a, 2017b). Consequently, it does not allow intensive NM or AI. If the primordial condition—the presence of active corpora lutea (CL)—was in place, photostimulated goats could attain the necessary conditions for synthetic prostaglandin analogues to be efficiently used and, at the same time, provide synchronous and fertile oestrus in breeding (Maia et al., 2017). Another important question is related to possible different efficiency responses among younger goats (nulliparous and primiparous), which have shown a more marked seasonality than multiparous ones under tropical conditions (Balara et al., 2019) after a period of photostimulation. In both conditions, the association

between active CL which can support oestrous synchronization and parity order needs to be investigated in goats subjected to oestrous induction by light programme.

The objective of this study was to assess, for the first time, the efficacy of an oestrous synchronization protocol with two cloprostenol administrations 11.5 days apart in photostimulated primiparous and multiparous dairy goats.

2 | MATERIAL 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 #9826011018) and was conducted according to the principles of the Brazilian Society of Laboratory Animal Science, which regulates conditions for trials involving animals.

2.2 | Location and experimental conditions

The study was carried out from June to December (winter to early summer), during the anestrus season, in Santo Antônio do Aventureiro (21°45'S and 42°48'W), Minas Gerais State, Brazil. All experimental goats were kept under an intensive farming system and fed with corn silage, with concentrate provided on demand (NRC, 2007). Mineralized salt and fresh water were offered *ad libitum*.

2.3 | Experimental animals, oestrous induction and synchronization

The experimental diagram is shown in Figure 1. The commercial dairy goat system under investigation had 115 adult mature females and four mature bucks. During the annual reproductive management programme, 50%–60% and 40%–50% of females mated during the breeding and non-breeding season, respectively, according to the milk demands of the market. A total of 56 Saanen females were selected ($n = 33$ multiparous and $n = 23$ primiparous), which were free from any reproductive disorder detectable by transrectal ultrasound (Maia et al., 2018), aged between 2 and 5 years, and producing an average 2.4 kg milk per goat daily in the final third of the lactation period (200–230 days lactation). All goats were subjected to a daily artificial photoperiod treatment consisting of 16 hr of light and 8 hr of darkness, starting 10 days after the winter solstice and lasting 60 days (D0 = June 30th and D60 = August 29th). A timer device (FR2Eletro®, Bivolt TM-BV2) was programmed to turn the light on

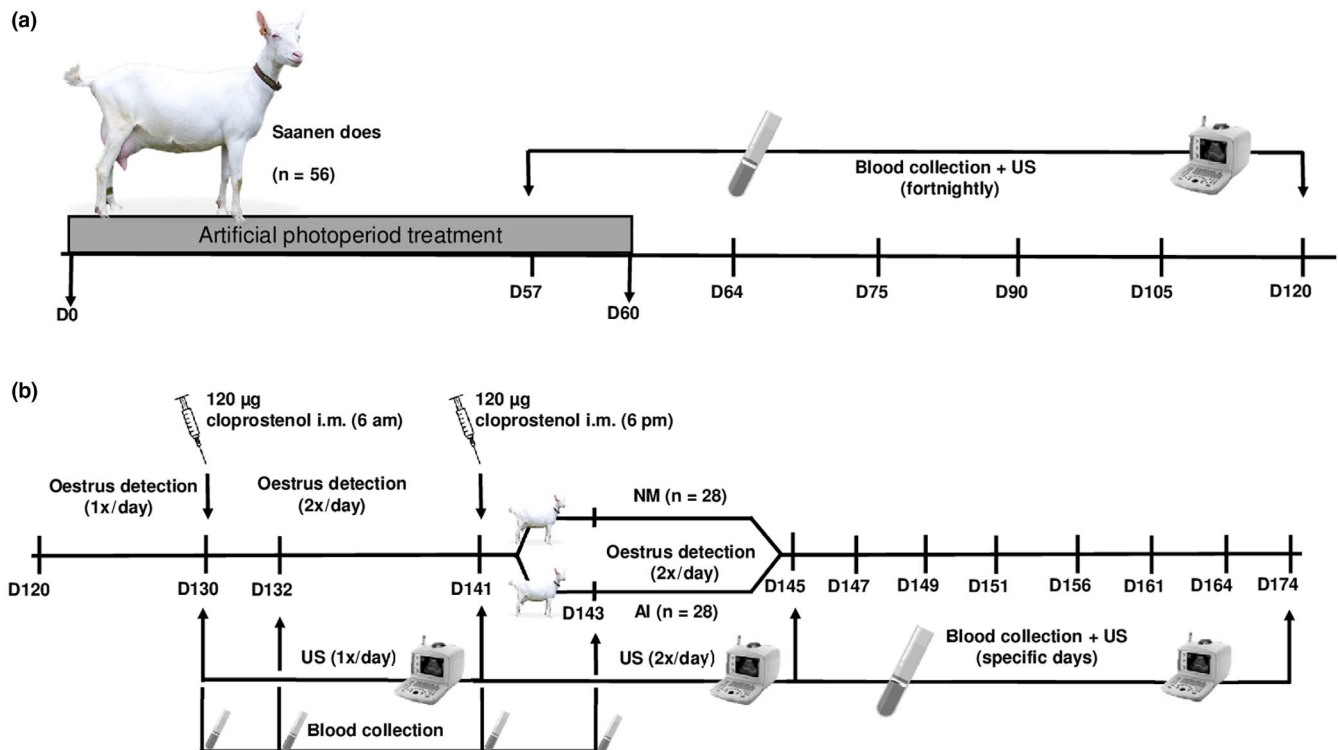


FIGURE 1 Schematic representation of the experiment design with (a) artificial photoperiod treatment (light programme) consisting of 16 hr of light and 8 hr of darkness, starting 10 days after the winter solstice (D0 = June 30th) and lasting 60 days (D60 = August 29th) followed by (b) oestrous synchronization with two doses of cloprostenol 11.5 days apart. Ultrasonography evaluation (US); blood collection for progesterone measurement; oestrous detections; breeding with artificial insemination (AI; $n = 28$) or natural mating (NM; $n = 28$) and oestrous cycle monitoring with final pregnancy diagnosis are indicated (see the text for details)

from 4 to 8 p.m. and again from 4 to 8 a.m. Following a similar previous study (Rodrigues, 1992), cloprostenol administration began on D130.

On D130 (6 a.m.) and D141.5 (6 p.m.) (11.5 days apart), all animals received two doses of 120 µg cloprostenol (Estron®, Agener União) i.m. (Bonato et al., 2019). The oestrus of all goats was monitored once daily from D120 to D130 and twice daily from D130 to D145.5, using photostimulated bucks.

2.4 | Animal breeding

Four bucks of proven fertility received the same light programming as the females. Seminal evaluation was carried out 7 days before breeding. Three bucks with adequate seminal levels ($\geq 80\%$ seminal progressive linear motility and $\leq 20\%$ seminal pathology), according to Brazilian College of Animal Reproduction (2013), and with fertility $> 60\%$ in the previous local natural breeding season were selected and used.

Because of the breeding plan, goats were equally allocated into two groups at D120 according to their body condition score (BCS; 1 = very thin and 5 = very fat; Villalquira, Gipson, Merkel, Goetsch, & Sahlu, 2007), parity order, and pedigree, and were subjected to either assisted NM (primiparous = 12 and multiparous = 16) or AI (primiparous = 11 and multiparous = 17). One primiparous goat from

the AI group was discarded due to clinical problems. Does were bred (1:6 buck:doe ratio) only at the beginning of oestrus. The male pens were located 80 m linearly from the female pens.

Goats in oestrus from the AI group were inseminated by Embrapa's transcervical technique (Fonseca et al., 2017a), using frozen-thawed semen of proven fertility from the Brazilian Breeding Plan for Dairy Goats and Progeny Test—The CapraGene® (Facó et al., 2011). The Flexible Time AI (FxAI) strategy (Bonato et al., 2019; Maia et al., 2017) was applied, whereby for goats presenting first oestrus at 24/36, 48 or 60 hr after the second cloprostenol administration, AI was performed 24, 18 and 10 hr later, respectively.

2.5 | US evaluation

US examinations were performed on 20 goats (primiparous: $n = 10$ and multiparous: $n = 10$) equally assigned to NM ($n = 10$) and AI ($n = 10$) groups using a portable device (Mindray®, M5Vet) with a 7.5 MHz linear rectal transducer suitable for use in small ruminants, with does in a standing position. Ovarian US examinations were performed every 15 days from D60 (the last day of the light treatment) to D130 (the first cloprostenol dose). Follicular dynamics were evaluated and recorded according to a previous study (de Castro, Rubianes, Menchaca, & River, 1999) every 12 hr from each cloprostenol administration to 12 hr after ovulation was confirmed

(D130–D134 and D141.5–145.5). Taking the second day after oestrus onset as day 0 of the oestrous cycle (D144-ovulation day), both B-mode and doppler evaluation were performed on the 3rd (D147-initial CL formation and detection), 5th (D149-CL confirmation), 7th (D151-CL functionality or early regression), 12th (D156-CL functionality and middle regression), 17th (D161-CL functionality and physiological regression), 20th (D164-CL functionality/maintenance; early pregnancy), and 30th (D174-CL functionality/maintenance; confirmation of pregnancy) days of the cycle. It was considered as premature luteal regression when it was initially visualized a luteal structure in formation in D147 followed by loss of luteal blood flow and luteal echogenicity in D149, D151 or D156 as previously described by Cosentino et al. (2018). Pregnancy was assessed 30 days after breeding, based on CL viability and the detection of at least one viable embryonic vesicle (embryo with heartbeats). All examinations were performed by the same technician, and B-Mode and colour Doppler settings were standardized and kept 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; Wall Filter [WF]: 75 KHz) (Cosentino et al., 2018).

2.6 | Blood sampling and progesterone assay

Blood samples were collected by jugular venipuncture into tubes without anticoagulant from does subjected to US examinations and on the same days as these examinations except for Days 57 and 64 for checking CL function/anestrus. Does were also sampled on the

days of cloprostenol administration (immediately before administration) to identify CL function as well as 2–2.5 days later to identify cloprostenol action on CL activity. These samples were always taken in the early morning and were 130 and 132 for the first and 141.5 and 143 for the second cloprostenol administration (Figure 1). After collection, blood samples were centrifuged at $1,500 \times g$ for 15 min. Then, serum was aspirated and stored at -20°C for hormonal assay. Serum P4 values were evaluated by a solid phase radioimmunoassay technique using commercial kits (ImmuChem, MP Biomedicals). Sensitivity and intra-assay coefficients were 0.05 ng/ml and 11%, respectively. All data were within the maximum and minimum point of the curve. A hormonal assay was performed on 16 selected animals in which no clinical problems had been detected during the experiment, and comprehended primiparous ($n = 8$) and multiparous ($n = 8$) does.

2.7 | Variables and statistical analysis

The recorded data included the following: oestrous response: number of females in oestrus/number of females exposed $\times 100$; time to oestrus: period (hr) between cloprostenol administration to time of first oestrous identification (onset of oestrus); duration of oestrus: period (hr) between first to last oestrous identification; period (hr) to ovulation: period from cloprostenol administration to ovulation; period from oestrus onset to ovulation; ovarian follicular diameter (mm); number of ovulation: number of ovulations/number of goats ovulating; serum progesterone concentration (ng/ml); percentage of goats with CL: goats with CL/goat evaluated by US $\times 100$;

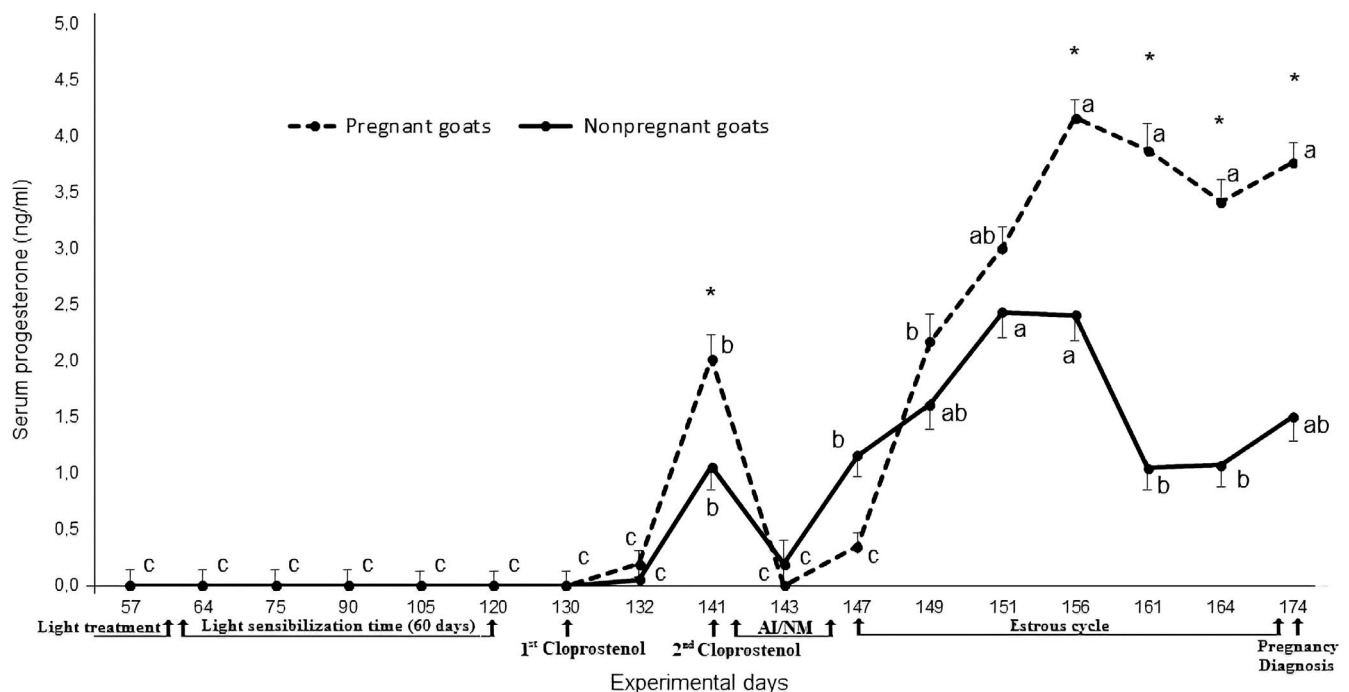


FIGURE 2 Mean values of serum progesterone concentrations in Saanen goats receiving light treatment for 60 days (D0–D60) that became pregnant ($n = 7$) or not ($n = 9$). ^{a,b,c}Means with different superscripts in the same line differed ($p < .05$; Friedman's test). *Means difference between groups (pregnant \times non-pregnant goats; $p < .05$; Mann-Whitney's test)

TABLE 1 Reproductive outcomes of photostimulated Saanen goats receiving two 120 µg of cloprostenol administration 11.5 days apart (% or Mean ± SEM)

End points	Cloprostenol administration	
	First dose	Second dose
Oestrous response (%)	27.3 ^a (15/55)	63.6 ^b (35/55)
Interval to oestrus (hr) [*]	26.4 ± 5.2 ^{Bb}	44.5 ± 1.9 ^{aA}
Duration of oestrus (hr)	30.1 ± 2.1	29.3 ± 1.6
Interval to ovulation (hr) [*]	97.0 ± 18.3 ^A	71.7 ± 2.1 ^B
Interval from oestrus onset to ovulation (hr) ^{**}	35.3 ± 12.4 ^A	28.9 ± 2.2 ^B
Diameter of largest follicle (mm) [*]	8.7 ± 0.4	8.0 ± 0.2
Diameter of second largest follicle (mm) [*]	6.8 ± 0.3	7.2 ± 0.2
Number of ovulations [*]	1.5 ± 0.1	1.8 ± 0.1

Note: () Number of animals.

^{a,b}Means with different superscripts within rows differed ($p < .05$;

Fisher's exact test- frequency and Mann-Whitney test-non-parametric). ^{A,B}Means with different superscripts within rows differed ($p < .05$; Bartlett's Test) for homocedasticity of variances.

^{*}Not compared in function of different intervals of oestrous monitoring.

^{**}Animals evaluated by ultrasonography ($n = 19$).

conception rate: goats pregnant/goats bred × 100; and pregnancy rate: goat pregnant/goats exposed × 100.

Data were analysed using a statistical program for statistical analysis (BioEstat[®]). Lilliefors and Bartlett tests were used to verify the normality and homoscedasticity of variables, respectively. Non-parametric unpaired variables (total and parity order reproductive indices) were submitted to the Mann-Whitney test. Non-parametric paired variables (P4) were submitted to the Friedman test to compare data over time. Categorical data (frequency rates) were assessed by Fisher's exact test. For all tests, $p < .05$ was considered significant and values from $p = .051$ to $p = .10$ were considered as tendencies.

3 | RESULTS

There was no interaction between parity order and P4 concentrations in does that became pregnant or not. Thus, P4 data were evaluated regardless of parity order and the mean P4 concentrations are shown in Figure 2. All animals showed P4 < 1.0 ng/ml from D57 to D130, confirming anestrus status. Half (8/16) of the females showed P4 > 1.0 ng/ml at the time of the second cloprostenol administration. From 16 goats evaluated, seven became pregnant. Non-pregnant goats included three goats bred and six goats not bred. Pregnant goats demonstrated greater P4 values at D141, D156, D161, D164 and D174 than non-pregnant ones. However, at D141 the difference found between the P4 production in the pregnant and non-pregnant group is related to the greater number of goats with luteal structure in the first when compared to the second one (71.4%; 5/7 vs. 33.3%;

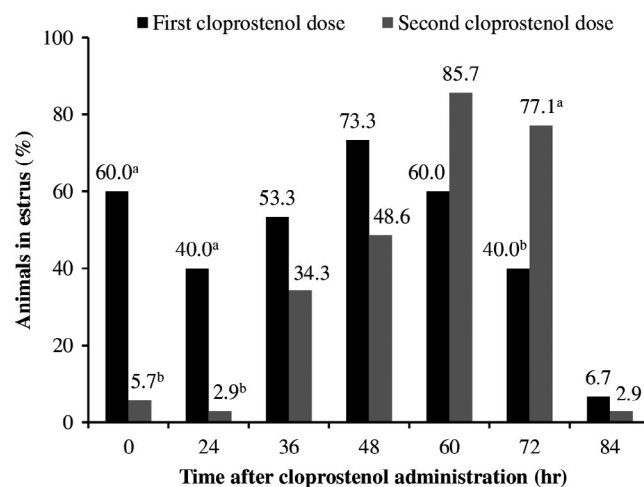


FIGURE 3 Frequency (%) of photostimulated goats in oestrus at the time and after of the first and second dose of cloprostenol 11.5 days apart. It not includes non-responsive does. ^{a,b}Frequencies with different superscripts differed ($p < .05$; Fisher's exact test) on the same time (hr)

3/9), and not due to low P4 production within the group itself. At D161 (20 days after the second dose or 17th day of the oestrous cycle), 66.6% (6/9) of non-pregnant animals presented a serum concentration of P4 > 1.0 ng/ml.

From D60 to D120, the majority (89%; 17/19) of goats scanned presented large follicles (5–8 mm) while the others (11%; 2/19) presented only medium-sized ones (3–5 mm). There was no CL (0%; 0/19) during this period.

From D120 to D129 (before the first cloprostenol injection), 29.1% (16/55) of the goats already presented oestrus. At D130, 16.4% (9/55) of the animals were in oestrus, differing ($p < .05$) from D141.5, when only 3.6% (2/55) of animals were in oestrus. Therefore, 45.4% (25/55) of goats showed previous oestrus or were in oestrus at the time of the first cloprostenol injection.

Regardless of parity order and breeding technique, the reproductive outcomes observed before the first cloprostenol injection and after both first and second cloprostenol administrations are shown in Table 1. Data related to distribution of oestrus onset after both cloprostenol injections are shown in Figure 3. The greatest percentage of animals in oestrus after the second application of cloprostenol was seen at 60 hr, with no difference ($p > .05$) between parity orders. After the first cloprostenol dose, the goats showed more dispersed oestrus than after the second dose. After the second dose, oestrus was more concentrated in the period from 48 to 72 hr.

Reproductive outcomes regarding the parity order of goats (primiparous or multiparous) and breeding management (NM or AI) are shown in Table 2. Regardless of the breeding management technique, primiparous showed a lower oestrous response and conception rate than multiparous goats (Table 2). As regards breeding techniques, conception rates were similar ($p > .05$) for NM (70.6% or 12/17) and AI (61.1% or 11/18). Only the time to oestrus differed ($p < .01$) between NM (51.3 ± 2.7 hr) and AI (40.2 ± 2.9 hr). Ovulation after both cloprostenol doses ($n = 10$) positively affected ($p < .05$)

TABLE 2 Reproductive outcomes of photostimulated Saanen goats of different parity orders receiving two 120 µg of cloprostenol administration 11.5 days apart (% or Mean ± SEM)

End points	Parity order						Overall
	Primiparous			Multiparous			
	NM	AI	Total	NM	AI	Total	
Oestrous response (%)	58.3 (7/12)	30.0 (3/10)	45.5 ^a (10/22)	75.0 (10/16)	88.2 (15/17)	75.8 ^b (25/33)	63.6 (35/55)
Interval to oestrus (hr)	48.3 ± 4.8	39.5 ± 8.0	45.0 ± 4.2	52.5 ± 3.3	40.3 ± 3.2	45.7 ± 2.6	45.6 ± 2.2
Duration of oestrus (hr)	33.6 ± 2.4	28.0 ± 4.0	31.5 ± 2.2	31.0 ± 4.0	28.0 ± 3.2	29.3 ± 2.5	29.8 ± 2.0
Interval from second cloprostenol administration to ovulation (hr) [*]	68.0 ± 6.9	68.0 ± 0.0	68.0 ± 3.1	80.0 ± 6.9	71.0 ± 3.0	74.9 ± 3.6	71.7 ± 2.5
Interval from oestrus onset to ovulation (hr) [*]	24.5 ± 0.0	28.5 ± 8.0	28.5 ± 5.1	30.5 ± 6.0	30.5 ± 6.0	30.0 ± 4.1	28.9 ± 2.9
Diameter of largest follicle (mm) [*]	8.2 ± 0.5	7.5 ± 0.6	7.9 ± 0.4	8.8 ± 0.9	7.7 ± 0.5	8.1 ± 0.5	8.0 ± 0.3
Diameter of second largest follicle (mm) [*]	7.4 ± 0.1	6.8 ± 0.0	7.2 ± 0.2	7.1 ± 0.0	7.2 ± 0.5	7.2 ± 0.4	7.2 ± 0.2
Number of ovulations [*]	1.7 ± 0.3	1.7 ± 0.7	1.7 ± 0.3	1.8 ± 0.3	2.0 ± 0.0	1.9 ± 0.1	1.8 ± 0.2
Goats with corpora lutea before second cloprostenol administration (%) [*]	40.0 (2/5)	50.0 (2/4)	44.4 (4/9)	40.0 (2/5)	80.0 (4/5)	60.0 (6/10)	52.6 (10/19)
Conception rate (%) ^{**}	85.7 (6/7)	33.3 (1/3)	70.0 (7/10)	60.0 (6/10)	66.7 (10/15)	64.0 (16/25)	65.7 (23/35)
Pregnancy rate (%) ^{**}	50.0 (6/12)	10.0 (1/10)	31.8 ^a (7/22)	37.5 (6/16)	58.8 (10/17)	48.5 ^b (16/33)	41.8 (23/55)

Note: () Number of animals.

^{a,b}Percentages within rows with different superscripts differed ($p < .05$; Fisher's exact test)

^{*}Animals evaluated by ultrasound ($n = 19$);

^{**}animals were bred by either natural mating (NM) or artificial insemination (AI).

the pregnancy rate (80.0%; 8/10). More multiparous compared to primiparous goats (54.5%, 18/33 vs. 18.2%, 4/22) exhibited oestrus after both injections. More primiparous compared to multiparous goats (54.5%, 12/22 vs. 12.1%, 4/33) did not exhibit oestrus after any injection (Table S1).

The lack of oestrous response after second cloprostenol administration (58.1%; 18/31) and consequently no breeding was the most prevalent cause of non-pregnancy in goats studied. Two goats in oestrus at the time of the second cloprostenol administration were not mated (6.5%; 2/31). Causes of non-conception in bred animals ($n = 11$) detected by US examinations included premature luteal regression ($n = 3$), hydrometra ($n = 1$) and persistent anovulatory follicle ($n = 1$), while no apparent reproductive disorder was seen in six animals.

4 | DISCUSSION

To our knowledge, this was the first study associating the artificial photoperiod treatment with cloprostenol administrations in dairy goats during the non-breeding season to obtain good results. We obtained encouraging results after the association of the most

effective and least artificial technique for inducing oestrus outside the breeding season—light programme—and the most genuine form of synchronizing oestrus in cyclic goats, the prostaglandin F2 α analogue. Important aspects of oestrous cycle control with more complex hormonal protocols are the residues in milk (Chemineau et al., 2007) and the environment (Gonzalez-Bulnes, Menchaca, Martin, & Martinez-Ros, 2020); reduced activities due to hormonal administrations and animal restraints (eCG and intravaginal device insertion and removal); and the risk of diminishing efficiency after successive use (eCG immune response; Baril et al., 1996). These factors are closely related to the technique proposed in the present study and encourage its use for synchronized oestrous induction in acyclic goats. This protocol is fully aligned and in accordance with the principles of the 'Clean, Green, and Ethical' technique to control reproduction in small ruminants (Gonzalez-Bulnes et al., 2020; Martin & Kadokawa, 2006; Vilanova et al., 2019).

From D60 to D120, most goats presented large dominant follicles. This finding supports the fact that follicular growth occurred until the last phase, even during the anestrus season, although ovulation did not occur, probably due to low LH pulsatility during this time, as demonstrated in sheep (Legan & Karsch, 1980). Similar follicular growth was previously reported in goats raised in lower

latitude (Cruz, Rondina, & Freitas, 2005). We may speculate that approaches such as socio-sexual stimulation (the male and female effect) or even flushing could be efficient tools to induce oestrus in such animals during that period.

When the second cloprostenol dose was administered, 50% (8/16) of animals presented serum P4 concentration <1.0 ng/ml. This result differed from that found by Maia et al. (2018) during the breeding season, when both nulliparous and multiparous goats presented P4 > 1.0 ng/ml at the time of the cloprostenol application. This finding could be explained by a parity order effect found in the current study because primiparous goats have a lower oestrous response to the light programme/cloprostenol protocol than multiparous ones. In this sense, the superior outcomes obtained by multiparous goats may be related to their longer life and local adaptation to the environmental conditions (Chemineau, Daveau, Cognié, Aumont, & Chesneau, 2004), which could reduce the influence on their endogenous rhythm (Hazlerigg, Andersson, Johnsson, & Lincoln, 2004). Nevertheless, such rhythm could still influence younger animals such as primiparous and nulliparous goats. Similarly, a more marked seasonality was recently demonstrated in nulliparous and primiparous goats than in multiparous ones under tropical conditions (Balara et al., 2019). Therefore, further studies are needed to improve reproductive outcomes in younger goats.

In the present study, the overall oestrous response was 64%. This rate was lower than the 97% obtained in goats after progestogen-based treatment (Pietroski, Brandão, Souza, & Fonseca, 2013) and similar to/lower than the 67%–84% reported after the use of similar light programme combined with progestogen/progesterone implants and the male effect (Pellicer-Rubio et al., 2008). However, it was higher than the 50% obtained by using melatonin implants for oestrous induction (Chemineau et al., 1992). Indeed, our intermediate overall oestrous response seems reasonable since we did not use any gonadotropin or other hormone that requires milk discharge, which is important for commercial dairy systems. This synchronized oestrous response (all goats in oestrus within 4 days of the second dose) was different from the 54 to 84 days period after light programming when only the artificial photoperiod technique was applied (Rodrigues, 1992). Therefore, the methodology proposed in this study allows the use of either intensive NM or AI. It should be emphasized that oestrus was checked only 4 days after cloprostenol administration, and 66.7% of the non-pregnant goats presented serum P4 values >1.0 ng/ml in all measurements after the second dose (D161, D164, D174). This result highlights that goats continued their cycle, allowing further breeding chances with no need of progestogen or gonadotropin. Thus, the number of animals in oestrus could be increased by extending the time of oestrous monitoring or by the permanent presence of the buck in the pen during this period.

In the breeding season, when the same protocol was applied (two doses of cloprostenol 11.5 days apart), greater oestrous response rates were obtained in dairy goats after the second dose: 88%–97% (Maia et al., 2017) and 84%–87% (Bonato et al., 2019). The first authors reported a near 30% greater oestrous response after

the second (88%) than after the first (59%) cloprostenol dose, similar to what was seen in the present study (27% vs. 64%). Possibly, the female–female effect (Rodríguez-Martínez et al., 2013) also played a role in this increase. It is important to highlight the oestrous synchronization obtained in the period from 36 to 60 hr after the second dose, which is interesting for AI programs. Although in the present study the interval to oestrus (45.6 hr) was slightly less than the 48–50 hr previously reported with the same synchronization treatment (Bonato et al., 2019; Maia et al., 2017), the interval to ovulation (71.6 hr) was similar to that reported in cyclic goats (71.7 hr). Considering that interval to ovulation is a more assertive variable, it can be suggested that if the cyclic condition is provided by light programme, reliable results can be obtained.

Interestingly, 94% of responding animals were in oestrus 36–60 hr after the second dose, which allows AI based on oestrous detection in the same day, applying the FxTAI strategy (Bonato et al., 2019; Maia et al., 2017). In Experiment 2, AI was conducted at 60 and 70 hr, respectively, for goats in oestrus 36 and 60 hr after the second cloprostenol dose, when most of the animals were still in oestrus. Hence, animals were inseminated at the end of oestrus (84 hr) and near ovulation (Fonseca et al., 2012). These data encourage further studies and suggest that light programme/cloprostenol provided synchronized oestrus which could support FxTAI in the non-breeding season. In addition, the possibility of breeding by NM those animals with either precocious oestrus (24 hr) or those who are in oestrus after the expected desirable time for AI (60 hr), as well as those who do not become pregnant after the first breeding, could increase overall flock fertility.

The overall conception (66%; goats bred) and pregnancy (42%; total goats) rates were affected by different factors. The most prevalent was the absence of oestrous response after the second cloprostenol administration at the expected time, which resulted in a lower pregnancy rate for primiparous goats, even though conception rates were similar in both parity groups. Other factors affecting pregnancy in the present study included reproductive disorders often detected in dairy goats. In fact, premature CL regression is one of the main reasons for subfertility in small ruminants in the transition period (Christensen, Haresign, & Khalid, 2014) and has already been reported in cyclic goats synchronized with two doses of cloprostenol (Fonseca et al., 2012), as well as hydrometra (Maia et al., 2017). These biological findings must be taken into account and should be studied in greater depth for a better understanding of all mechanisms involved.

5 | CONCLUSIONS

Artificial light programme followed by two cloprostenol administrations allowed the efficient induction of synchronized oestrus and ovulation in Saanen goats in the non-breeding season, especially in multiparous goats. Good pregnancy rates were reached, whether an NM or AI breeding management programme was used. Overall, the current study opens a new perspective to reach oestrous synchronization in goats during the non-breeding season without using

progestogen/gonadotropin and can be implemented by dairy goat farmers in the non-breeding season.

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CONFLICTS OF 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.

AUTHORS CONTRIBUTION


JFF involved in elaboration of the hypothesis and experimental design. MMN, MFAB, FZB and JFF discussed and approved the experimental design. MMN, MFAB, IOC and CGES: involved in conception and study design, data collection, statistics and creation of the first draft. FZB performed the hormonal analyses. FZB, JFF, RVO and JMGSF involved in critical evaluation of the manuscript. In addition, all authors contributed to the writing, revision and approval of the final version of the manuscript.

DATA AVAILABILITY

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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