

## Hormonal Protocols for the Synchronization and Induction of Synchronized Estrus in Dairy Ewes Kept under Tropical Conditions

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### ABSTRACT

**Background:** Lacaune is an important sheep breed and shows reproductive seasonality which affects dairy product supply. The most common hormonal protocols are based on progesterone-releasing devices in order to reach reproductive activity. Due to international consumer demand for natural, green, and clean methods, such issues encourage the minimal or absent use of synthetic hormones in livestock. In this sense, the present study tested the efficiency of induction and estrus synchronization protocols in lacaune ewes, considering: application interval of synthetic prostaglandin at the breeding season; length of synthetic progesterone exposure; and eCG dose at the outbreeding season.

**Materials, Methods & Results:** In study 1: 52 ewes were submitted to estrus synchronization protocol using two doses of d-cloprostenol 7 or 9 days apart. In study 2: 47 ewes were submitted to estrus induction protocol using an intravaginal device containing 60 mg of medroxyprogesterone acetate maintained for 6, 9, or 12 days. In study 3: 46 ewes were submitted to synchronized estrus induction protocol using intravaginal progesterone device for six days. On the fifth day, d-cloprostenol and 300 IU or 400 IU of eCG were administered. For all studies, signs of estrus beginning/ending were recorded and natural mating was performed. After 30 days, transrectal ultrasound was performed for pregnancy diagnosis. In study 1, groups presented similar rate of estrus behavior (78.8%) and pregnancy (average 75.6%). Meanwhile, compared to  $G_{9days}$  ( $37.8 \pm 7.2$  h), interval between the second dose of prostaglandin and beginning of estrus was smaller in  $G_{7days}$  ( $31.5 \pm 7.8$  h). No difference was found among variables studied in study 2 ( $P > 0.05$ ), nevertheless the SD for the estrus duration was shorter ( $P < 0.05$ ) in  $G_{6days}$  and  $G_{12days}$  when compared with the  $G_{9days}$ . Equally, the SD for the interval between sponge withdrawal and beginning and end of estrus was shorter ( $P < 0.05$ ) in  $G_{12days}$  when compared with the  $G_{9days}$ . The pregnancy rate, at 30 days, was 71.1%. With regards to study 3, there was no effect of parturition order on sexual behavior between experimental groups ( $P > 0.05$ ). Equally, no differences were found between variables studied ( $P > 0.05$ ). The pregnancy rate was 52.2% and number of concepts was  $1.5 \pm 0.6$ .

**Discussion:** This paper provides important data on estrus synchronization protocols in dairy sheep kept under tropical conditions. In study 1, both d-cloprostenol protocols were efficient in synchronizing estrus of dairy ewes and reached a fair pregnancy rate. Therefore, it was demonstrated that a d-cloprostenol treatment was a viable alternative to a progestogen treatment, commonly used for estrus synchronization in ewes even during the breeding season. In study 2, all protocols (6, 9 and 12 days of exposure to synthetic progesterone) were efficient in inducing and synchronizing estrus in dairy ewes during the outbreeding season. Therefore, considering not only its efficiency but also the reduction of damage to the vaginal microbiota, the authors strongly encourage the use of the 6-day synthetic progesterone exposure protocol. The third study showed that it is possible to reduce the eCG dose (400 to 300 IU) in dairy ewes while maintaining protocol efficiency, saving 25% of the farmer spending, and avoiding high doses that are related to immune resistance and lower progressive fertility response to artificial insemination. In conclusion, the present study verified the efficiency of different hormonal protocols applied for dairy Lacaune ewes kept under tropical conditions, demonstrating that it is possible to decrease: (i) the interval between prostaglandin applications for 7 days apart during the breeding season; (ii) the length of exposure to synthetic progesterone for 6 days; and (iii) the eCG dose to 300 IU during the outbreeding season.

**Keywords:** dairy sheep, equine corionic gonadotropin, progestogens, prostaglandins, breeding management.

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## INTRODUCTION

Lacaune is an important dairy breed for sheep farming and shows reproductive seasonality which affects the supply of dairy products throughout year [3]. The most common hormonal protocols, by being based on progesterone-releasing devices in order to reach reproductive activity, mimic the luteal phase by long-term treatments (12-14 days). Moreover, other hormones, such as equine chorionic gonadotropin (eCG), can stimulate the final stages of follicle growth and ovulation, improving fertility results [1,7,8,15,16,19]. Nevertheless, it has been reported that long-term treatments can cause vaginitis and affect viability of ram sperm, as well as prolong lifespan of large follicles, releasing an older oocyte with less chance of being fertilized [9,10,21]. On the other hand, studies have demonstrated that short-term treatments can induce cyclic activity during seasonal anestrus in meat ewes [13,19,20].

Due to international consumer demand for natural, green, and clean methods, such issues encourage the minimal or absent use of synthetic hormones in livestock farming [11,17]. In this sense, protocols based on prostaglandins, associated or not with gonadotrophins, are effective in promoting estrus synchronization during the breeding season in meat sheep under tropical conditions, presenting lower cost and easy application, besides not being related to tissue residues and vaginitis [2,4,5,19]. Therefore, the study aimed to test the efficiency of induction and estrus synchronization protocols in dairy ewes, considering: (i) application interval of synthetic prostaglandin (d-cloprostenol) at the breeding season; (ii) length of synthetic progesterone exposure; and (iii) eCG dose at the outbreeding season.

## MATERIALS AND METHODS

### *Experimental conditions*

All studies were located in Rio de Janeiro, Brazil, performed on a dairy sheep farm (22°24'14"S). The experimental ewes were maintained in a semi-intensive system under natural photoperiod. Ewes had access to pasture (*Melinis minutiflora* and *Brachiaria humidicola*) and received 0.4 kg/ewe daily of concentrate (homemade mixture with 14% crude protein), 5.0 kg/ewe of chopped grass (*Pennisetum purpureum*), water, and mineralized salt *ad libitum*. Previously all studies, no one ewe had any reproductive disorder indicated by clinical or ultrasound scan.

### *Study 1*

The first study was conducted during the breeding season (May). A total of 52 dry Lacaune ewes (37 pluriparous and 15 nulliparous) received two doses of 37.5 µg of d-cloprostenol (Prolise®)<sup>1</sup> i.m. at 7 (G<sub>7days</sub>: n = 25; 18 pluriparous and 12 nulliparous; 2.6 ± 1.2 years old; Body condition score [BCS]: 3.0 ± 0.3) or 9 (G<sub>9days</sub>: n = 27; 19 pluriparous and 13 nulliparous; 2.8 ± 1.6 years old; BCS: 3.0 ± 0.2) days apart. Twenty-four h after the second dose, ewes were subjected to mating by 6 fertile Lacaune rams (3.2 ± 1.6 years old; BCS: 3.1 ± 0.2) twice a day until they showed no more estrus signs. Ultrasonographic pregnancy diagnosis was conducted in all ewes at 30 days after mating. Between the second dose and onset/end of estrus signs, estrus duration, estrous rate, gestation rate, and the birth order effect on protocol performance, the intervals were assessed.

### *Study 2*

The second study was conducted during the outbreeding season (August). A total of 47 dry pluriparous Lacaune ewes (3.2 ± 1.8 years old; body weight: 53.2 ± 8.6 kg; BCS: 3.0 ± 0.4) received intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (Progespon®)<sup>2</sup>. The sponges were maintained for 6 (G<sub>6days</sub>: n = 14), 9 (G<sub>9days</sub>: n = 16), or 12 (G<sub>12days</sub>: n = 17) days. One day before its removal, 37.5 µg of d-cloprostenol<sup>1</sup> i.m. and 300 IU of eCG (Novormon®)<sup>2</sup> i.m. were administered in all ewes. The ewes were subjected to mating by 6 fertile Lacaune rams after sponge removal (3.3 ± 1.6 years old; BCS: 3.1 ± 0.1) twice a day until they showed no more estrus signs. At 30 days after mating, ultrasonographic pregnancy diagnosis was conducted in all ewes. The intervals between the sponge removal and onset/end of estrous signs, estrus duration, estrus rate and gestation rate were assessed.

### *Study 3*

The third study was conducted during the outbreeding season (September). A total of 46 dry Lacaune ewes (24 pluriparous and 22 nulliparous) received a natural progesterone device (CIDR®)<sup>3</sup> maintained for 6 days. One day before its removal, 37.5 µg of d-cloprostenol<sup>1</sup> i.m. and 300 IU (G<sub>300IU</sub>: n = 24; 12 pluriparous and 11 nulliparous; 2.6 ± 1.6 years old; BCS: 3.0 ± 0.3) or 400 IU (G<sub>400IU</sub>: n = 22; 12 pluriparous and 11 nulliparous; 2.4 ± 1.3 years old; BCS: 3.0 ± 0.2) of eCG<sup>3</sup> i.m. were administered. After device

removal, ewes were subjected to mating by 6 fertile Lacaune rams ( $3.3 \pm 1.6$  years old; BCS:  $3.2 \pm 0.1$ ) twice a day until they showed no more estrus signs. At 30 days after mating, ultrasonographic pregnancy diagnosis was conducted in all ewes. The estrus rate, gestation rate, number of concepts, and the birth order effect on protocol performance were assessed.

#### Ultrasonography

Ultrasonography assessments were performed using portable equipment (SonoScape® S6 V.)<sup>4</sup> with a transrectal linear probe of 7.5 MHz, coupled with a PVC structure for use in small ruminants. Ewes were maintained in a standing position, and 20 mL of carboxymethylcellulose gel was placed into the rectum with a syringe. Ultrasound was carried out prior to all studies in order to check the ewes' cyclical activity (diestrus or anestrus; by the corpus luteum view) and genital tract health. Pregnancy was confirmed on Day 30 based on the detection of at least one viable embryonic vesicle (embryo with heartbeats) within the uterine horn using B-Mode US. Equally, all exams were performed by the same technician.

#### Statistical analysis

Data were analyzed using a statistical program (BioEstat 5.3.®)<sup>5</sup>. Lilliefors and Bartlett test were used to verify the normality and homocedasticity of variables, respectively. The F variance test was used to examine the differences in variability among different experimental groups. Non-parametric variables from study 1 and 3 were submitted to the Mann-Whitney U-test to compare groups. Non-parametric variables from study 2 were submitted to the Kruskal-Wallis' and Dunn's tests to compare means. Categorical data were assessed by the Fisher's exact test. For all tests,  $P < 0.05$  was considered significant.

## RESULTS

### Study 1

There was no effect of parturition order on sexual behavior between experimental groups ( $P > 0.05$ ). In this sense, reproductive findings were presented regardless of parturition order (Table 1). Likewise, the estrus rate, estrus duration, interval between the second dose of d-cloprostenol to the end of estrus, and pregnancy rate did not differ between experimental groups ( $P > 0.05$ ; Table 1). Meanwhile, the interval between the second application of d-cloprostenol to the beginning of estrus was shorter ( $P < 0.05$ ) in G<sub>7days</sub> ( $31.5 \pm 7.8$  h; mean  $\pm$  standard deviation [SD]) when compared to G<sub>9days</sub> ( $37.8 \pm 7.2$  h; mean  $\pm$  SD).

### Study 2

No difference was found among variables studied ( $P > 0.05$ ; Table 2), nevertheless the SD for the estrus duration was shorter ( $P < 0.05$ ) in G<sub>6days</sub> and G<sub>12days</sub> when compared with the G<sub>9days</sub>. Equally, the SD for the interval between sponge withdrawal and beginning and end of estrus was shorter ( $P < 0.05$ ) in G<sub>12days</sub> when compared with the G<sub>9days</sub>. The mean estrus rate was 95.7%. The mean estrus duration was  $29.6 \pm 11.0$  h (mean  $\pm$  SD). The mean interval between sponge removal and estrus presentation was  $34.7 \pm 11.7$  h (mean  $\pm$  SD). The mean interval between estrus beginning and ending was  $64.3 \pm 9.1$  h (mean  $\pm$  SD). The pregnancy rate, at 30 days, was 71.1%.

### Study 3

There was no effect of parturition order on sexual behavior between experimental groups ( $P > 0.05$ ). In this sense, reproductive findings were presented regardless of parturition order (Table 3). Equally, no differences were found between variables studied ( $P > 0.05$ ; Table 3). The average estrus rate was 80.4%. At 30 days after mating, the pregnancy rate was 52.2% and number of concepts was  $1.5 \pm 0.6$  (mean  $\pm$  SD).

**Table 1.** Reproductive outcomes (mean  $\pm$  standard deviation [SD]) from Lacaune ewes (n = 52) submitted to estrus synchronization using d-cloprostenol seven or nine days apart during the breeding season under tropical conditions.

Group	Estrus rate (%)	Estrus Duration (h)	Interval between 2 <sup>nd</sup> PG dose and		Pregnancy rate* (%)
			Estrus beginning (h)	Estrus ending (h)	
G <sub>7days</sub>	80.0 (20/25)	$40.2 \pm 12.5$	$31.5 \pm 7.8^b$	$71.7 \pm 8.05$	85.0 (17/20)
G <sub>9days</sub>	77.8 (21/27)	$37.1 \pm 8.4$	$37.8 \pm 7.2^a$	$74.9 \pm 7.08$	66.7 (14/21)
Total	78.8 (41/52)	$38.6 \pm 10.6$	$34.7 \pm 8.1$	$73.4 \pm 7.6$	75.6 (31/41)

<sup>a,b</sup>Different superscripts within columns indicate difference by Mann-Whitney U-test ( $P < 0.05$ ). \*Pregnancy rate adjusted by ewes that showed estrus and were mated.

**Table 2.** Reproductive outcomes (mean  $\pm$  SD) from Lacaune ewes (n = 47) submitted to induction of synchronized estrus using an intravaginal progesterone-releasing device protocol for six, nine or 12 days in the outbreeding season under tropical conditions

Group	Estrus rate (%)	Estrus Duration (h)	Interval between sponge withdrawal and		Pregnancy rate* (%)
			Estrus Beginning (h)	Estrus ending (h)	
G <sub>6days</sub>	85.7 (12/14)	32.0 $\pm$ 7.8 <sup>b</sup>	33.0 $\pm$ 9.6 <sup>ab</sup>	65.0 $\pm$ 6.9 <sup>ab</sup>	83.3 (10/12)
G <sub>9days</sub>	100 (16/16)	30.9 $\pm$ 14.9 <sup>a</sup>	36.0 $\pm$ 15.5 <sup>a</sup>	66.9 $\pm$ 12.7 <sup>a</sup>	56.3 (9/16)
G <sub>12days</sub>	100 (17/17)	26.6 $\pm$ 8.2 <sup>b</sup>	34.8 $\pm$ 9.2 <sup>b</sup>	61.4 $\pm$ 5.3 <sup>b</sup>	76.5 (13/17)
Total	95.7 (45/47)	29.6 $\pm$ 11.0	34.7 $\pm$ 11.7	64.3 $\pm$ 9.1	71.1 (32/45)

\*Pregnancy rate adjusted by ewes that showed estrus and were mated. <sup>a,b</sup>Different superscripts within columns indicate SD difference by F test ( $P < 0.05$ ).

**Table 3.** Reproductive outcomes from Lacaune ewes (n = 46) submitted to induction of synchronized estrus using 300 IU or 400 IU of eCG in the outbreeding season under tropical conditions.

Group	Estrus rate (%)	Pregnancy rate* (%)	Number of concepts
G <sub>300IU</sub>	79.2% (19/24)	68.4 (13/19)	1.3 $\pm$ 0.6
G <sub>400IU</sub>	81.8% (18/22)	61.1 (11/18)	1.6 $\pm$ 0.5
Total	80.4% (37/46)	65.0 (24/37)	1.5 $\pm$ 0.6

\*Pregnancy rate adjusted by ewes that showed estrus and were mated.

## DISCUSSION

This paper provides important data on estrus synchronization protocols in dairy sheep kept under tropical conditions. In study 1, both d-cloprostenol protocols (seven or nine days apart) were efficient in synchronizing estrus of dairy ewes and reached a fair pregnancy rate (75.6%). Equally, in the study conducted by Almeida *et al.* [4], they found a comparable pregnancy rate (75.0%) using the same protocol in meat ewes under similar conditions. Although, Fierro *et al.* [5] in their study reported lower pregnancy rates (51.5%) using two PG doses administered 14 days apart in meat ewes under temperate conditions. Also, Hasani *et al.* [7] in their study indicated the use of eCG after the second prostaglandin (protocol 12 days apart) in order to improve the pregnancy rate (87.5%) in meat ewes under tropical conditions.

It is important to highlight that our results demonstrated that 7 days prostaglandin interval promoted a faster onset of estrus signs (31.5 h), which was similar to previous results already reported [4] (36.5 h) under similar conditions, as well as with a lower dispersion in ovulation. Such intervals are shorter than those observed in protocols with prostaglandin injections 12 days apart (~44 h) [2,5]. Therefore, it was demonstrated that a d-cloprostenol treatment with two doses seven

days apart in dairy ewes under tropical conditions was a viable alternative to a progestogen treatment, commonly used for estrus synchronization in ewes even during the breeding season [14]. Moreover, it was showed that, unlike progestogen treatment [10], vaginal mucus from ewes treated with prostaglandins for estrus synchronization did not affect functionality and viability of sheep semen, and presented a 2.63 lower cost, compared to the progestogen use [2].

In study 2, all protocols (6, 9 and 12 days of exposure to synthetic progesterone) were efficient in inducing and synchronizing estrus in dairy ewes during the outbreeding season, with an overall 95.7% of estrus response and 71.1% of pregnancy. Equally, an interesting finding related to the G<sub>9days</sub> showing greater dispersion of sexual behavior when mainly compared to G<sub>12days</sub>, although pregnancy rates did not differ. This result may be justified by the distinct moment of follicular growth in which experimental groups were associated with the total time to progestogen exposure at time of sponge removal. Regarding conception rates, Ungerfeld and Rubianes [20] using MAP and FGA-impregnated sponge treatment during 6 days in meat ewes described similar rates of 62.5% and 67.4%, respectively. Meanwhile, Teixeira *et al.* [19] compared treatments with 6, 9 and 12 days of MAP-impregnated



sponge and described lower conception rates (~ 34%). Still, other studies used progestogen-based protocols during seven days in the breeding season, with similar conception rates (75%) in dairy ewes [14] or greater conception rates (87%) in meat sheep [21]. In addition, short-term exposure to progestogens has also been described using different devices (CIDR®)<sup>3</sup> and with good conception rates being reached [13-15,20,21]. So, a short-term treatment with progestogens proved to be equally effective for inducing estrus behavior, preovulatory LH discharge, and ovulation in sheep [13], resulting in a good concept rate, probably due to the ovulation of newly recruited growing follicles [21]. Therefore, considering not only its efficiency but also the reduction of damage to the vaginal microbiota, we strongly encourage the use of the 6-day synthetic progesterone exposure protocol [14].

The third study showed that it is possible to reduce the eCG dose (400 to 300 IU) in dairy ewes while maintaining protocol efficiency, saving 25% of the farmer spending, and avoiding high doses that are related to immune resistance and lower progressive fertility response to artificial insemination [18]. Adding eCG greatly increases the protocol cost, although it denotes a key role to the increase of the reproductive efficiency of progestogen and prostaglandins protocols [15]. Also, due to the long metabolization of eCG, it takes an extended time to eliminate it. Such issues have led to a decrease in eCG doses in the past years. Some authors proposed a 500 IU of eCG in hormonal protocols for hair sheep [2,6]. Best results were achieved using protocols with 5 days of progesterone exposure with reduction of the eCG dose to 400 IU in ewes in

the outbreeding season [15]. In short, dairy ewes kept under tropical conditions reach a good reproductive response by using 300 IU of eCG dose.

## CONCLUSIONS

The present study verified the efficiency of different hormonal protocols applied for dairy Lacaune ewes kept under tropical conditions, demonstrating that it is possible to decrease: (i) the interval between d-cloprostenol applications for 7 days apart at the breeding season; (ii) the length of exposure to synthetic progesterone for 6 days; and (iii) the eCG dose to 300 IU at the outbreeding season. All findings are clearly relevant in terms of cost reduction with hormonal protocols, as well as improving animal welfare and minimizing residues in animal production.

## MANUFACTURERS

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**Ethical approval.** This research was approved by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (protocol 855/16) and conducted under the ethical principles of the Sociedade Brasileira de Ciência em Animais de Laboratório. All applicable international, national, and/or institutional guidelines for the care of animals were followed.

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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