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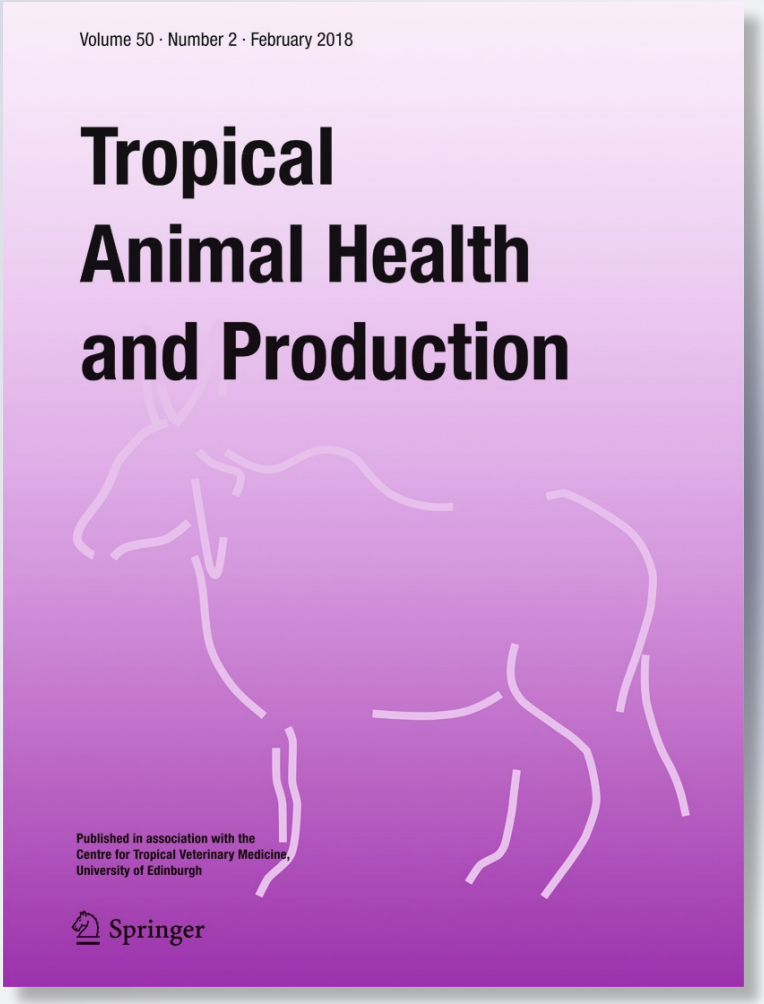

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REGULAR ARTICLES

Use of two doses of cloprostenol in different intervals for estrus synchronization in hair sheep under tropical conditions

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Abstract This study evaluated the effect of two doses of prostaglandin at different intervals on reproductive parameters of crossbred ewes. In Experiment 1, 30 ewes received two doses of 120 μg cloprostenol at 7 ($G_{7\ days}$), 9 ($G_{9\ days}$), or 11.5 ($G_{11.5\ days}$) days apart. Ultrasound assessments were performed from the first and second cloprostenol administration for 5 days or ovulation detection. Estrus signs were checked by a teaser male. Plasma progesterone concentration was measured before each cloprostenol dose. In Experiment 2, 95 ewes were allocated into the same treatments and after the second dose, ewes in estrus were mated. At 30 days after breeding, pregnancy diagnosis was conducted and prolificacy was evaluated at lambing. In Experiment 1, at the first cloprostenol administration, 50% of ewes had an active CL and all showed estrus. At the second administration, 66.7% of ewes had an active CL and one did not present estrus. There was no difference ($P > 0.05$) after the second dose for as follows: overall estrous response (90%), interval from cloprostenol administration to estrous onset (42.0 ± 4.9 h), estrus duration (31.5 ± 2.1 h), ovulation rate (100.0%), and number of ovulations (1.5 ± 0.3). In Experiment 2, both pregnancy and prolificacy rates were similar ($P > 0.05$) for $G_{7\ days}$ (73.3; 145%), $G_{9\ days}$ (75.9; 125%), or $G_{11.5\ days}$ (75.9; 145%), leading to an overall pregnancy rate of 75.0% (66/88) and prolificacy rate of 137%. Therefore, the three treatments proposed were able to promote high pregnancy and prolificacy rates in crossbred ewes.

Keywords Cyclicity · Ovine · Native breed · Progesterone · Prostaglandin

Introduction

Sheep farming has experienced an outstanding growth in developing countries and provides protein of high-biological value that is derived from their milk and meat. Among the sheep breeds raised in Brazil, Santa Inês stands out because of its adaptability to tropical conditions, maternal ability, prolificacy, and sexual precocity (Rocha et al. 2004; Balaro et al. 2014). Moreover, Dorper is a fast-growing meat-producing sheep which adapts well to Brazilian environments due to its African origin. Thus, Dorper × Santa Inês crossbred production is becoming more usual among farmers. The use of reproductive biotechnologies as estrus synchronization in this species has aided the growth of genetic and productive gains, as well as organize mobs for mating and lambing period throughout the year.

Conventional treatments for estrus synchronization usually associate progesterone, prostaglandin, and gonadotropins. Nevertheless, repeated use of gonadotropins such as eCG has resulted in low-pregnancy rates due to humoral immune response in small ruminants. Furthermore, possible alternatives to allow a "clean, green, and ethical" estrus synchronization are essential nowadays. One strategy is to use only prostaglandins for this purpose (Abecia et al. 2012). It is well documented that prostaglandin is only effective during the breeding season due to the need of having an active corpus

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luteum in the ovary (McCracken et al. 1972). Despite the known photoperiod phenomenon in small ruminants, our group previously demonstrated that in Brazilian Southeast, Santa Inês sheep has overall low seasonality (Balaro et al. 2014). Therefore, the definition of a great strategy using only prostaglandins is crucial.

Prostaglandin administration either in a single dose or in two doses may be used to synchronize cyclic ewes. The initial studies proposed a 9-day interval between both doses (Reviewed by Abecia et al. 2012). Our group usually applied a 7-day interval (Texeira et al. 2016), when most ewes will be in a precocious phase of interovulatory cycle and thus will have a CL sensible to the action of a new dose of prostaglandin (Menchaca et al. 2004). However, the use of an interval of 11.5 days allows the development of a new follicular wave from the first cloprostenol administration and luteolysis in the middle of luteal phase, when the CL is still able to respond to the prostaglandin action, even in the case of early regression of corpus luteum, as previously demonstrated in goats (Maia et al. 2017). This event, although often neglected in sheep, has considerable importance. Thus, these data justify a study designed to perform a direct comparison among shorter, traditional, or longer intervals.

The aim of the present study was to evaluate the effect of the administration of two doses of prostaglandin at 7, 9, or 11.5 days apart on sexual behavior, ovulatory parameters, and pregnancy rate of Dorper × Santa Inês crossbred ewes during the breeding season.

Material and methods

Experimental conditions

This study was approved by the Animal Care Committee of Fluminense Federal University (protocol number 452), and it was conducted under the principles of Brazilian Society of Laboratory Animal Science. The study was carried out in the rural area of Cachoeiras de Macacu located in the state of Rio de Janeiro (latitude 22°27′ S, longitude 43°39′ W, 577 m of altitude) during the breeding season. According to Köppen, the climate is a tropical hot-humid type, with temperatures throughout the year ranging from 15 to 30 °C and an annual rainfall ranging from 2.000 to 2.600 mm^3 concentrated mostly in the summer.

In both experiments, the ewes were maintained in a semi-intensive system under natural photoperiods with access to pasture during the day and shelter at night. Chopped elephant grass (*Pennisetum purpureum*) and 250 g per animal of a concentrate with 18% crude protein were offered once a day, with water and mineralized salt (Ovinofos®, Tortuga, São Paulo, Brazil) ad libitum.

Experiment 1

This experiment was conducted during the month of March, characterized as breeding season. A total of 30 nulliparous or nonlactating pluriparous Santa Inês ewes were randomly allocated into three experimental treatments according to their category, age, body weight (BW), and condition score (BCS), respectively: $G_{7\ days}$: n = 10; 47.7 ± 8.8 kg and BCS = 30.0, 60.0, and 10.0% from 2.5, 3.0, and 3.5, respectively; $G_{9\ days}$: n = 10; 44.9 ± 7.5 kg and BCS = 40.0, 40.0, and 20.0% from 2.5, 3.0, and 3.5, respectively; and $G_{11.5\ days}$: n = 10; 46.3 ± 5.4 kg and BCS = 30.0, 50.0, and 20.0% from 2.5, 3.0, and 3.5, respectively. All ewes received two doses of 120 μg cloprostenol i.m. (Estron®, Agener União, São Paulo, Brazil) at 7 ($G_{7\ days}$), 9 ($G_{9\ days}$), or 11.5 ($G_{11.5\ days}$) days apart.

Ultrasound assessments were performed every 12 h from the first cloprostenol administration (Day 0) for 5 days or the occurrence of ovulation and again from the second dose (Day 7, Day 9, or Day 11.5) for 5 days or the occurrence of ovulation. The ewes were subjected to a teaser male after each cloprostenol administration, twice a day (6:00 am and 6:00 pm), for 5 days until they showed no more estrus signs. Plasma progesterone concentration was measured in all ewes immediately before each cloprostenol dose.

Experiment 2

This experiment was conducted during the months of April and May, also characterized as breeding season. A total of 95 nulliparous or nonlactating pluriparous Dorper × Santa Inês crossbred ewes were randomly allocated into the same three treatments according to their category, age, and condition score (BCS), respectively: $G_{7\ days}$: n = 33; BCS = 8.3, 75.0, and 16.7% from 2.5, 3.0, and 3.5, respectively; $G_{9\ days}$: n = 31; BCS = 4.3, 73.9, and 21.7% from 2.5, 3.0, and 3.5, respectively; and $G_{11.5\ days}$: n = 31; BCS = 20.0, 60.0, and 20.0% from 2.5, 3.0, and 3.5, respectively. The ewes were divided into four mobs of approximately 24 females each, with intervals of 1 week per mob, to respect male resting. Ewes were subjected to a teaser male after the second dose, every 12 h (6:00 am and 6:00 pm) for 5 days or until they showed no more estrus signs. Ewes in estrus were mated by four fertile rams for approximately equal numbers of ewes from each treatment, with a ram:ewe ratio about ≤ 1:6. At 30 days after breeding, ultrasonographic pregnancy diagnosis was conducted in all ewes and prolificacy was evaluated at lambing.

Ultrasonography

During the experiments, ultrasonography assessments were performed (SonoScape®, S6 V, Shenzhen, China) with a transrectal linear probe of 7.5 MHz, coupled with a PVC structure for the 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. The follicular diameter and position, the number of follicles, and CL were recorded. The day of ovulation was defined as the day when the largest follicle, previously identified, was no longer detected. Double ovulation was defined when the second largest follicle at the time of ovulation was greater than 4.5 mm in diameter.

Plasma progesterone concentrations

Blood samples were collected in the morning by jugular venipuncture, into vacuum tubes containing EDTA for progesterone analysis. The samples were immediately chilled at 5 °C, centrifuged (3000*g* for 15 min), and then the plasma was separated into microtubes and frozen at − 20 °C. The progesterone analysis was performed at the Hormonal Dosage Laboratory, Animal Reproduction Unit, Fluminense Federal University. The progesterone concentrations were determined by commercial solid phase radioimmunoassay (RIA) kit (MP Biomedicals, LLC, Diagnostics Division, Orangeburg, NY, EUA), according to the manufacturer's instructions. The assay sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 10%. In addition, all data were within the maximum and minimum points of the curve.

Statistical analysis

For statistical analysis, the software Bioestat® 5.3 (Ayres et al. 2007) was used. The significance level adopted for these analyses was 5%. Data are presented as Mean ± SD. The quantitative variables were tested as its normality by the Lilliefors test and, then, submitted to one-way ANOVA and Tukey test [estrus duration (interval from the first to last acceptance of mounting); interval from first and second dose to estrous onset; interval from first and second dose to ovulation; interval from estrous onset to ovulation; number of ovulations per ewe; largest and second largest follicle diameter; plasma progesterone concentration]. The qualitative variables were subjected to Fisher Exact Test [BCS; estrous response rate; ovulation rate (number of ewes with confirmed ovulation/number of ewes evaluated by ultrasonography × 100); pregnancy rate]. Additionally, the Bartlett test was applied to analyze the variance of the interval from cloprostenol administration to estrous onset obtained after each treatment.

Results

Experiment 1

Three animals (two from $G_{7\ days}$ and one from $G_{11.5\ days}$) were removed became ill and were removed after the second dose. The results for estrus behavior and ovulation after the first and second cloprostenol administration are showed in Table 1. After the first dose, one ewe from $G_{11.5\ days}$ presented estrus signs but did not ovulate. After the second dose, all animals that ovulated presented estrus. Of the ewes in estrus after the first and second dose, respectively, 45.4% (10/22) and 59.3% (16/27) were initially identified in estrus in the morning and 54.5% (12/22) and 40.7% (11/27) in the evening, with no difference ($P > 0.05$) among treatments and period of time. At 12 h after both cloprostenol administrations, a total of five ewes showed signs of estrus. Out of these, three (two from $G_{7\ days}$ and one from $G_{11.5\ days}$) after the first and two (one from $G_{9\ days}$ and one from $G_{11.5\ days}$) after the second dose. At 60 h after the first dose, estrus behavior was observed in 100% of responding animals from $G_{9\ days}$ and $G_{11.5\ days}$, whilst at 72 h in all $G_{7\ days}$ ewes. However, at 60 h after the second

Table 1 Sexual behavior parameters of crossbred ewes after receiving the first or second dose of cloprostenol (PGF) with 7, 9, and 11.5 days apart for estrus synchronization (Mean ± SD)

Parameter	$G_{7\ days}$		$G_{9\ days}$		$G_{11.5\ days}$		Total	
	First	Second	First	Second	First	Second	First	Second
Estrus response (%)	80.0	80.0	80.0	100.0	60.0	90.0	73.3	90.0
Interval to estrus (h)	36.5 ± 21.2	36.5 ± 6.2	32.5 ± 10.0	45.8 ± 18.9	39.0 ± 16.4	43.7 ± 16.7	36.0 ± 3.3	42.0 ± 4.9
Estrus duration (h)	28.5 ± 11.0	31.5 ± 8.9	37.5 ± 7.7	33.6 ± 12.4	24.0 ± 13.1	29.3 ± 12.2	30.0 ± 6.9	31.5 ± 2.1
Interval from PGF to ovulation (h)	66.4 ± 23.5	56.8 ± 6.2	59.0 ± 15.8	67.9 ± 24.4	75.0 ± 6.2	78.7 ± 9.4	66.7 ± 7.8	67.8 ± 11.0
Interval from estrus to ovulation (h)	29.9 ± 8.7	20.3 ± 6.1[a]	26.5 ± 8.1	25.5 ± 12.2[a,b]	30.5 ± 6.0	35.0 ± 20.2[b]	29.0 ± 2.2	26.9 ± 7.5
Ovulation (%)	100.0	100.0	100.0	100.0	83.3	100.0	94.4	100.0
Number of ovulations	1.1 ± 0.4	1.8 ± 0.7	1.4 ± 0.7	1.2 ± 0.4	1.7 ± 0.5	1.4 ± 0.5	1.4 ± 0.3	1.5 ± 0.3
Diameter of the largest follicle (mm)	5.7 ± 1.1	6.4 ± 0.5	6.2 ± 0.5	6.1 ± 0.9	5.8 ± 0.5	6.2 ± 0.7	5.6 ± 0.7	6.1 ± 0.3
Diameter of the 2nd largest follicle (mm)	4.9 ± 0.7	6.0 ± 0.9	5.9 ± 1.1	6.0 ± 0.0	5.4 ± 0.4	6.0 ± 0.8	5.4 ± 0.5	6.1 ± 0.1

($P > 0.05$) Different superscripts in the same row indicate significant difference after the second dose. *Experiment 1

dose, 100% ($G_{7\ days}$), 70% (G_{9days}), and 87.5% ($G_{11.5\ days}$) were in estrus. Regardless of the treatment, the highest concentration of estrus occurred between 48 and 60 h, both after the first (77.3%; 17/22) and second (66.7%; 18/27) dose. Females from $G_{7\ days}$ entered in estrus between 36 h (37.5%) and 48 h (62.5%). After the second dose, females from $G_{7\ days}$ presented the shortest ($P < 0.05$) interval from estrus to ovulation when compared to the other treatments (Table 1).

In order to evaluate the estrus synchronization treatment, females were allocated into two different groups—that presented or not estrus signs—and also according to the progesterone concentrations measured at the first and second dose, as ewes with active CL ($P_4 > 1$ ng/mL) and non-active CL ewes ($P_4 < 1$ ng/mL) and data are presented in Table 2. Out of the eight animals that did not demonstrate estrus after the first cloprostenol dose, only two did not show estrus after the second dose. Considering these two animals, one had subluteal concentrations in both moments and the other presented active CL in the moment of second dose. One female presented active CL in the first administration and subsequently showed estrus whilst in the second dose, even demonstrating supraluteal progesterone concentrations; she did not come into estrus.

Experiment 2

There was no difference ($P > 0.05$) after the second dose among groups $G_{7\ days}$, $G_{9\ days}$, or $G_{11.5\ days}$ ewes for as follows: estrous response [91% (30/33), 94% (29/31), or 94% (29/31), respectively], estrus duration (33.2 ± 13.3, 33.9 ± 12.5, or 36.0 ± 10.6 h), interval from cloprostenol administration to estrous onset (33.4 ± 11.1, 36.5 ± 8.7, or 38.5 ± 7.4 h), interval from cloprostenol administration to the end of estrus (66.6 ± 13.3, 70.4 ± 10.4, or 74.5 ± 9.3 h) and number of ovulations (1.41, 1.23, or 1.45). There were no differences ($P > 0.05$) for pregnancy rates taking into consideration all ewes in each group or those that were previously detected in estrus, respectively, for $G_{7\ days}$ [66.7 (22/33) or 73.3 (22/30)], $G_{9\ days}$ [71.0 (22/31) or 75.9 (22/29);] or $G_{11.5days}$ [71.0 (22/31) or 75.9 (22/29)], leading to a high-pregnancy rate [75.0% (66/88) or 69.5% (66/95)]. Prolificacy rates at kidding were similar ($P > 0.05$) among groups, averaging as follows: $G_{7\ days}$ [141%], $G_{9\ days}$ [123%], or $G_{11.5\ days}$ [145%], leading to an overall prolificacy rate of 137%.

There was no interaction between category and treatment. A total of seven ewes did not show estrus, four pluriparous and three nulliparous. Regardless of the treatment, there was no difference ($P > 0.05$) between nulliparous and pluriparous ewes on the following end points, respectively: interval from cloprostenol administration to estrous onset (35.4 ± 9.5; 39.1 ± 6.7 h) and estrous duration (34.3 ± 11.2; 34.6 ± 12.6 h). When different categories were compared, nulliparous reached 85.7% (24/28) and pluriparous 70.0% (42/60) of pregnancy rate.

Discussion

The results of this study showed that all three hormonal treatments were efficient to synchronize estrus in Dorper × Santa Inês crossbred ewes in the beginning of breeding season in Southeastern Brazil. Similar estrous response rates were detected among ewes from all treatments after both doses. As expected, a lower percentage (73.3 versus 90.0%) was obtained after the first or the second dose, respectively. This is easily explained since the first dose was administered in a random phase of estrous cycle and thus a number of ewes were not in diestrus and were not able to support luteolysis. The estrus response rates obtained in this study are in accordance to the literature, both after the first (Acritopolou and Haresign 1980; Texeira et al. 2016) and the second dose (Sozbilir et al. 2006; Silva et al. 2010; Texeira et al. 2016). It is well reported the necessity of previous exposure to progesterone so ewes can demonstrate estrus signs due to the increase in estradiol by

Table 2 Percentage of ewes that presented or not estrus according to the presence of active corpus luteum (CL) in the moment of the first (Day 0) or second cloprostenol administration (Day 7, Day 9, or Day 11.5)

		First		Second	
Treatment	CL	Total (%)	Estrus (%)	Total (%)	Estrus (%)
$G_{7\ days}$	+	60.0 (6/10)	100.0 (6/6)	62.5 (5/8)	100.0 (5/5)
	−	40.0 (4/10)	50.0 (2/4)	37.5 (3/8)	66.7 (2/3)
$G_{9\ days}$	+	60.0 (6/10)	100.0 (6/6)	80.0 (8/10)	100.0 (8/8)
	−	40.0 (4/10)	50.0 (2/4)	20.0 (2/10)	100.0 (2/2)
$G_{11.5\ days}$	+	30.0 (3/10)	100.0 (3/3)	55.6 (5/9)	80.0 (4/5)
	−	70.0 (7/10)	42.8 (3/7)	44.4 (4/9)	75.0 (3/4)
Total	+	50.0 (15/30)	100.0 (15/15)	66.7 (18/27)	94.4 (17/18)
	−	50.0 (15/30)	46.7 (7/15)	33.3 (9/27)	77.8 (7/9)

() Number of animals. *Experiment 1

preovulatory follicle (Bartlewski et al. 2011). Considering that the experiment started in the beginning of the breeding season, possibly few animals were still in the transition. This may be the reason why two animals did not present estrus after either the first or second dose.

The interval to estrus after the first dose was on average 36 h, similar to 37.7 h previously reported by Acritopoulou and Haresign (1980) and 39.5 h by Texeira et al. (2016). The interval to estrus after the second dose was on average 42.0 h, and this number is quite wide-ranging among studies. Rubianes et al. (2003) and Texeira et al. (2016) recorded 40.6 and 36.1 h after administering prostaglandin in 7-day interval, Acritopoulou et al. (1978) obtained 38.8 h after using 9-day interval and lastly Oyediji et al. (1990) reported 41.7 h after applying 11-day interval. At 12 h after both doses, five ewes were in estrus and it is difficult to affirm if those animals were already in estrus or if it was an answer from the treatment. Estrus duration after the second cloprostenol administration also did not differ among groups and was on average 31.5 h, similar to 30.0 h described by Mobini et al. (2002) and inferior to 39.1 h, reported by Texeira et al. (2016). Thus, treatments used in the present study did not affect interval to estrus or its duration as compared with results from previous reports. It is important to highlight that the frequent use of a teaser ram to aid estrus detection may have influenced the interval to estrus as well as its duration (Rosa and Bryant 2002).

Although there was no statistical difference between the average size of the largest follicle of each ovary, the $G_{7\ days}$ showed the greatest (NS) follicular diameter among the groups. It is known that a longer or shorter interval between the second dose and onset of estrus is also related to the final follicular diameter. Leyva et al. (1998) affirm that increased plasma concentrations of progesterone promote greater follicular turnover, shortening the length of a follicular wave, and favoring the development of a new one. High-progesterone concentrations reduce LH pulsatility, anticipating follicular atresia and decreasing the contribution of this gonadotrophin to the final follicular growth, but also increase the release of more glycosylated isoforms of FSH, allowing this hormone to reach the threshold necessary to promote emergence of a new wave (Bartlewski et al. 2011). Therefore, follicles subjected to low-progesterone concentrations have larger diameter and greater persistence than those subjected to high-progesterone concentration environments.

No differences were observed among protocols regarding ovulation rate but it was noted that the $G_{7\ days}$ presented the lowest dispersion of the interval from the second application to the occurrence of ovulation and from the onset of estrus to the occurrence of ovulation. This fact may have a great importance when planning to apply timed Artificial Insemination. It is noteworthy that there was an anticipation of the ovulation time. Probably these animals were closer to the follicular dominance period or already had a dominant follicle, whilst ewes receiving $G_{11.5\ days}$ and $G_{9\ days}$ could still be in the early follicular wave. Rubianes et al. (2003) and Contreras-Solís et al. (2009) found intervals of 60.8 and 61.1 h, respectively, for protocols based on 7-day interval. These values are slightly higher than those found in this study. Acritopoulou et al. (1978) found 73.1 h and Haresign and Acritopoulou (1978) 72.9 h in protocols with 9-day interval between doses. The standard deviation and coefficient of variation for both variables were the lowest for $G_{7\ days}$ compared to the other treatments. Therefore, if we consider a further use of timed Artificial Insemination, the appliance of the 7-day interval appears to be quite interesting, due to minimum range of variation.

Minton et al. (1991) stated that plasma values of progesterone below 1 ng/mL can characterize estrus or anestrus phases, while values greater than 3 ng/mL characterize the diestrus phase or pregnancy. Therefore, it is possible to infer that the two animals which did not respond to both applications of cloprostenol were possibly in seasonal anoestrus, eventhough one of them had progesterone value above 1 ng/mL (1.88 ng/mL). One ewe belonging to $G_{7\ days}$, responsive only after the first dose, presented 0.06 ng/mL of progesterone at the time of the second dose, besides a CL with morpho-structural features not compatible with active CL, suggesting this CL was already under regression process. According to Armstrong and Evans (1983), one of the probable causes of premature regression of CL can be a too quick follicular development. Thus, ovulation of follicles occurs in the granulosa cells which have not acquired a necessary maturity for optimal luteinization in response to the LH surge. Therefore, the phenomenon is associated with low-progesterone concentrations between the third and sixth day of the estrous cycle and early outbreak of luteolytic cascade (Lassoued et al. 1997).

It is well known that pregnancy rates are lower in prostaglandin-based protocols when compared with females in natural estrus (Fierro et al. 2011). However, in the current study, pregnancy rates were considerably high (75%), with no differences among treatments. These rates are slightly higher than those reported in other studies using two injections of cloprostenol. Fierro et al. (2011) reported pregnancy rates of 63% and prolificacy rate at 30 days of 1.27 and Boland et al. (1978) described 65% of pregnancy rate for the groups treated with two doses in a 9-day interval. Reproductive failures can occur at any time after estrus induction with prostaglandins. The prolificacy rate at birth in this study was higher than those reported by other authors. For $G_{7\ days}$ and $G_{11.5\ days}$, the rates were 141%, while for the $G_{9\ days}$ was 123%. Viñoles et al. (2009) applied a 9-day interval treatment and obtained a prolificacy rate of 105% at birth. Perhaps the positive result in the present study is due to the large number of animals or the breed used.

Although the use of two doses of cloprostenol in a 7-day interval led to lower dispersion at ovulation time, it is

reasonable to affirm that the three treatments proposed were able to promote high pregnancy and prolificacy rates in crossbred ewes in the beginning of the breeding season.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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