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REGULAR ARTICLES

Effect of eCG in a short-term synchronization treatment on ovarian status, estrus synchrony, and ovulation in dairy goats managed under tropical conditions

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Abstract

The aim of this study was to assess the need of using eCG on short-term estrus synchronization protocol in nulliparous (NUL) and multiparous (MULT) dairy goats during the breeding season. Alpine (n = 20), Nubian (n = 20), and Saanen (n = 16) goats received 60 mg medroxyprogesterone acetate intravaginal sponges for 6 days plus 30 μg d-cloprostenol and 200 IU eCG (G-eCG, n = 28) or saline (G-Control, n = 28) 24 h before sponge removal. The NUL and MULT goats of each breed were equally assigned into the two treatments. Transrectal ultrasonography was used to evaluate ovulatory parameters, and teaser goats were used for estrus detection every 12 h from sponge removal to ovulation. eCG did not affect ($P > 0.05$) estrus response (~86%), diameter of ovulatory follicles (~6.8 mm), and number of ovulations (~1.6). Nevertheless, eCG led to earlier ($P < 0.05$) ovulation (G-eCG = 65.1 and G-Control = 73.2 h) and increased ($P < 0.05$) the ovulation rate (G-eCG = 96.4% and G-Control = 67.9%). In the absence of eCG, no differences regarding reproductive parameters ($P > 0.05$) were found between parity orders. Alpine MULT goats underwent a superior ($P < 0.05$) number of ovulations (2.2) in comparison to NUL goats (1.3). In conclusion, the exclusion of eCG from short-term estrus synchronization protocol did not interfere with estrus response but decreased the ovulation rate.

Keywords Reproduction · Hormone · Does · Nulliparous · Multiparous · Gonadotropin

Introduction

Brazil has achieved great advances in goat milk production. In fact, Alpine, Nubian, Toggenburg, and Saanen goats raised in Brazil are able to reach similar milk yields as their counterparts all over the world, particularly those raised within the specialized and confined dairy herds located in the southeast region of the country (Lôbo et al. 2017). This improvement was strongly supported by CapraGene®, a Brazilian program for genetic improvement, implemented in 2005 (Facó et al. 2011). As a strategy for genetic improvement, assisted reproductive technologies (ART), such as estrous cycle control and artificial insemination (AI), are used to support progeny tests in dairy goat herds. Those ART commonly rely on synchronization protocols based on intravaginal progesterone or progestogen-releasing devices combined with cloprostenol and equine chorionic gonadotropin (eCG) (Abecia et al. 2012; Lopez-Sebastián et al. 2014). Due to its LH- and FSH-like activity (Combarnous et al. 1984), eCG has been used for estrous cycle control in ruminant females (Baril et al. 1996; Cavalcanti et al. 2011; Mahdavi-Roshan et al. 2020). Nevertheless, because it is a heavily glycosylated glycoprotein hormone, eCG administration leads to humoral immune responses, resulting in lower fertility rates in goats inseminated at a fixed time, after repeated treatments (Hervé et al. 2004). In addition, there is a growing concern about equine welfare issues involved in the production of this

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hormone, due to collection of large blood volumes from pregnant mares and abortion induction (Vilañova et al. 2019).

Disadvantages of the eCG use highlight the need of developing viable estrus synchronization protocols without this hormone. Recent studies reported encouraging results in the reproductive parameters assessed in goats and sheep. Ewes either receiving or not eCG administration during the breeding season maintained the same rates of estrus behavior, preovulatory LH surge, and ovulation, despite differences in the interval of these events (Uriol et al. 2019). However, better fertility rates were reported without eCG (Martinez-Ros et al. 2018). Encouraging rates of synchronous estrus induction after association of light program and cloprostenol, without eCG, were reported in anestrous Saanen goats (Netto et al. 2020). A negative effect of eCG on pregnancy rates was observed in Polwarth ewes subjected to a short-term protocol for estrus synchronization in the breeding season (Viñoles et al. 2001). Despite ethical (Vilañova et al. 2019) and practical exigency concerns regarding its use in acyclic goats (Netto et al. 2020), eCG continues to be used irrespective of the reproductive status, and there is no comparative study of its use during the breeding season and related effects on females with a different parity order. The efficiency of short-, mid-, and long-term estrus synchronization protocols combining progestogens, cloprostenol, and eCG (300 to 400 IU) was previously demonstrated in dairy goats during the breeding season (Martemucci and D'Alessandro 2011). However, to our knowledge, similar protocols without this gonadotropin have never been assessed in goats.

We hypothesized that the administration of eCG in short-term progestogen treatments during the breeding season is dispensable and brings no extra benefits in dairy goats being prepared to AI, regardless of the parity order. Thus, the aim of this study was to assess the need of using eCG on short-term estrous synchronization protocol in nulliparous (NUL) and multiparous (MULT) dairy goats during the breeding season.

Material and methods

Time and location

This study was conducted from February to March (beginning of the breeding season), in commercial herds at two different locations: Espírito Santo do Pinhal (22° 57′ S latitude and 46° 58′ W longitude) and Florestal (20° 45′ S latitude and 42° 51′ W longitude), Brazil.

Animals and synchronization protocol

A total of 56 goats of three different breeds (n = 20 Alpine, n = 20 Nubian, and n = 16 Saanen) with body condition score varying from 2.5 to 3.5 (1 to 5 range; Villaquiran et al. 2004) were selected. Both NUL (n = 30) and MULT (n = 26) goats of each breed were equally assigned into two treatments according to use or not of eCG. All MULT goats were in the final third of lactation. The estrus synchronization protocol used was previously described by Fonseca et al. (2017). On Day 0 (D0), goats received 37.5 μg d-cloprostenol (Veteglan®, Callier SA, Barcelona, Spain) via latero-vulvar, and an intravaginal progestogen sponge containing 60 mg medroxyprogesterone acetate (MAP; Progespon®, Zoetis, São Paulo, Brazil) was inserted and kept in place until D6. On D5, goats received either 200 IU eCG (Novormon 5000®, Schering Plough Animal Health, São Paulo, Brazil; G-eCG) or saline solution (G-Control) im 24 h before sponge removal (Fig. 1).

Ovarian ultrasound evaluation and estrus detection

Antral follicles (≥ 3 mm) were detected by transrectal B-mode ultrasonography (7.5 MHz transducer; KX 2000G Vet1, Kaixin, Xuzhou, China). The evaluation was performed by the same experienced operator every 12 h (0600 and 1800 h) from sponge removal to ovulation or up to 96 h after. The day of ovulation was recorded when the preovulatory follicle(s) was no longer detected (Souza et al. 2011). At the same interval, estrous behavior was assessed with six teaser bucks.

End points and statistical analysis

The following end points were assessed: estrus response (% of does that showed estrus), interval from sponge removal to estrus onset (h), interval from estrus onset to ovulation (h), interval from sponge removal to ovulation (h), ovulation rate (% of does that ovulated from those subjected to hormonal treatment × 100), number of ovulations (number of follicles ovulated per female), and diameter of ovulatory follicle (mm). The results are described as mean ± S.E.M, and categorical results are presented as percentages.

For statistical analysis, the Bio Estat 5.0 (Belém, Brazil) and IBM SPSS Statistics (version 19) software were used, and a 95% confidence interval was considered. Shapiro-Wilk test was used for normality and Levene's test for homogeneity of variance assessments. To evaluate the interaction between treatments and parity order, the General Linear Model (GLM) was applied. Parametric variables were subjected to Student's t-test. Non-parametric variables were analyzed by Mann-Whitney test, chi-square test, or Fisher's Exact test.

Results

There was no interaction ($P > 0.05$) between eCG treatment and parity order in all the studied parameters. Reproductive parameters of Alpine, Nubian, and Saanen goats from both

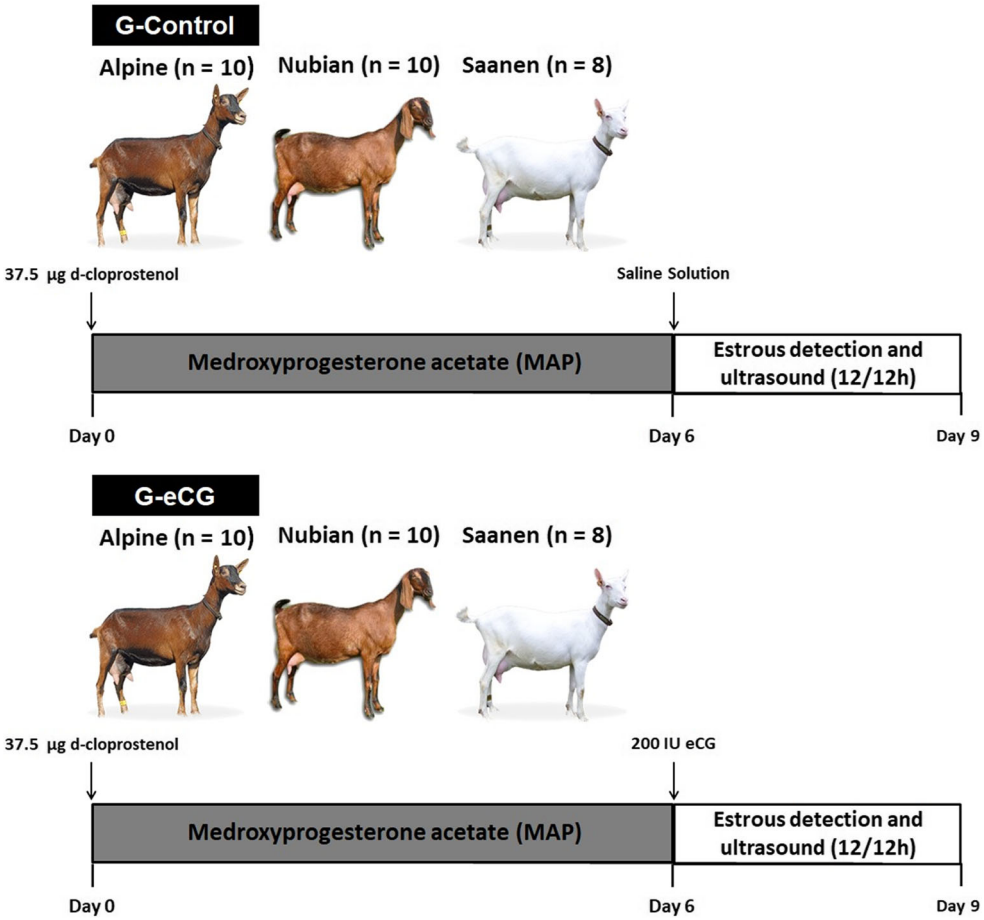

Fig. 1 Schematic representation of the experimental design of estrus synchronization with 37.5 µg d-cloprostenol at day 0, intravaginal progestogen sponge containing 60 mg medroxyprogesterone acetate starting in day 0 and kept until day 6, and saline solution in G-control versus 200 IU eCG in G-eCG, followed by estrus detection and ovarian ultrasound evaluation at every 12 h until day 9 (see the text for details)

parity orders, obtained with or without eCG administration, are shown in Table 1. Ovulation rates were similar ($P > 0.05$) between both experimental groups in Alpine and Nubian goats but superior ($P < 0.05$) in Saanen goats from G-eCG compared with G-Control. Regardless of the breed and parity order, overall ovulation rate was superior ($P < 0.05$) in goats treated with eCG compared with G-Control. Overall, the interval from sponge removal to ovulation was shorter ($P < 0.05$) in G-eCG, in comparison with G-Control goats.

Regardless of the breed, within G-eCG, NUL goats had a shorter ($P < 0.05$) interval from sponge removal to estrus onset, while MULT goats had a shorter ($P < 0.05$) interval from estrus onset to ovulation (Table 2). In addition, MULT goats had a shorter interval from sponge removal to ovulation in G-eCG, when compared to G-Control ($P < 0.05$). Regardless of the breed and hormonal treatment, overall interval from sponge removal to estrus onset was longer ($P < 0.05$) in MULT compared with NUL goats.

Discussion

The present study assessed reproductive parameters in NUL and MULT goats after short-term estrus synchronization protocol either with or without eCG, during the breeding season. Dairy goat breeds managed under Brazilian tropical conditions were used. For the best of our knowledge, this is the first study including three main dairy goat breeds (Alpine, Nubian, and Saanen goats) managed under tropical conditions and subjected to the same estrus synchronization protocol. No differences were found in estrus response in both NUL and MULT receiving or not eCG; despite that, eCG increased the ovulation rate.

The high estrus response in both treatments shows that synchronous estrus may be successfully achieved with short-term protocols without eCG. Similar results were previously reported in ewes (Viñoles et al. 2001; Martinez-Roz et al., 2018; Uriol et al. 2019) and does (Menchaca et al. 2007; Hashemi and Safdarian 2017) subjected to short- or long-term protocols, with or without eCG. The shorter overall interval from sponge removal to ovulation observed in goats treated with eCG corroborates with previous results in Alpine goats (Menchaca et al. 2007). These findings are supported by eCG's LH-like effect, which induces the final growth of preovulatory follicles and advances the preovulatory LH surge and ovulation, but with no effect on follicle final dimension (Menchaca et al. 2007; Uriol et al. 2019).

Table 1 Reproductive parameters (mean ± S.E.M. or %) of cyclic nulliparous (NUL) and multiparous (MULT) Alpine, Nubian, and Saanen goats subjected to 6-day progestogen and cloprostenol-based estrus synchronization protocol with (G-eCG) or without (G-Control) 200 IU equine chorionic gonadotropin (eCG) im administered 24 h before device removal

End points	Alpine		Nubian		Saanen		Total		NUL		MULT	
	G-eCG	G-Control	G-eCG	G-Control	G-eCG	G-Control	G-eCG	G-Control	G-eCG	G-Control	G-eCG	G-Control
Estrus response (%)	80.0 (8/10)	80.0 (8/10)	100.0 (10/10)	70.0 (7/10)	75.0 (6/8)	62.5 (5/8)	85.7 (24/28)	71.4 (20/28)	80.0 (12/15)	60.0 (9/15)	92.3 (12/13)	84.6 (11/13)
IREO (h)*	46.0 ± 5.4	53.5 ± 5.7	34.8 ± 3.3	36.0 ± 3.1	44.0 ± 5.1	52.8 ± 6.4	40.8 ± 2.7	47.2 ± 3.2	34.3 ± 2.1^{A}	41.8 ± 3.1	47.3 ± 4.6^{B}	51.6 ± 5.5
IEOV (h)**	27.0 ± 4.8	26.0 ± 4.5	25.2 ± 2.8	30.9 ± 2.0	22.3 ± 2.4	30.0 ± 0	25.1 ± 2.0	28.6 ± 1.9	29.3 ± 2.6^{A}	28.8 ± 2.7	20.8 ± 2.6^{B}	28.4 ± 2.9
IROV (h)***	64.8 ± 2.0	72.0 ± 3.1	67.0 ± 2.5	73.9 ± 2.0	63.7 ± 5.9	76.0 ± 6.5	65.1 ± 1.9^{a}	73.2 ± 1.9^{b}	64.7 ± 2.7	69.0 ± 2.3	65.5 ± 2.9^{a}	76.7 ± 2.6^{b}
Ovulation rate (%)	100.0 (10/10)	100.0 (10/10)	100.0 (10/10)	70.0 (7/10)	87.5 (7/8)^{a}	25.0 (2/8)^{b}	96.4 (27/28)^{a}	67.9 (19/28)^{b}	86.7 (13/15)	60.0 (9/15)	100.0 (13/13)	77.9 (10/13)
DOF (mm)****	7.2 ± 0.2	7.4 ± 0.3	6.7 ± 0.2	6.3 ± 0.2	6.5 ± 0.2	6.6 ± 0.3	6.8 ± 0.1	6.8 ± 0.2	6.7 ± 0.2	7.1 ± 0.3	6.8 ± 0.1	6.6 ± 0.2
Number of ovulations	1.7 ± 0.3	1.8 ± 0.3	1.2 ± 0.1	1.4 ± 0.2	2.0 ± 0.2	2.5 ± 0.3	1.6 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	1.3 ± 0.2	1.7 ± 0.2	2.1 ± 0.2

$^{a, b}$ Differ within rows between the hormonal treatments (G-eCG vs. G-Control) ($P < 0.05$)

$^{A, B}$ Differ within rows between the parity orders (NUL vs. MULT) within the same hormonal treatment ($P < 0.05$)

( )Number/proportion of animals

* IREO, interval from sponge removal to estrus onset; ** IEOV, interval from estrus onset to ovulation; *** IROV, interval from sponge removal to ovulation; **** DOF, diameter of ovulatory follicle

Regarding ovulation rate, a positive effect was observed upon the use of eCG in Saanen breed, whereas in Alpine and Nubian goats, similar results were observed in both treatments. These results indicate no need for eCG in the latter breeds, and the reason for such controversial results among breeds is unknown. In earlier studies, eCG had no effect on ovulation rate in Alpine goats (Menchaca et al. 2007), whereas curiously, either positive (Martinez-Roz et al., 2018), negative (Viñoles et al. 2001), or even no effect (Uriol et al. 2019) were reported in different sheep breeds. The addition of eCG in the present study did not influence the size and number of ovulatory follicles, similarly to previous studies using 5-day protocols in does (Menchaca et al. 2007) and ewes (Martinez-Roz et al., 2018; Uriol et al. 2019). Moreover, a lower eCG dose (200 IU) was used in the current study compared to previous reports (300 to 600 IU) (Baril et al. 1996; Roy et al. 1999; Greyling and Van Der Nest 2000; Martemucci and D'Alessandro 2011).

A shorter interval from sponge removal to estrus onset was observed in NUL, in comparison to MULT, regardless if they received eCG or not. With the removal of exogenous progestogen, LH frequency increases, and LH stimulates follicular growth and synthesis of estrogens, which are responsible for eliciting estrus behavior. One explanation for this earlier occurrence of estrus is that NUL does might have had higher circulating estradiol concentrations, not explained by the number of ovarian follicles growing, as observed in this study, but possibly due to a lower rate of steroid metabolism. This occurrence has been shown in cattle (Sartori et al. 2002), and in dairy goats submitted to a similar estrus synchronization protocol, we have previously reported greater plasma progesterone concentrations in NUL than in MULT (Souza et al. 2011). Alternatively, in NUL, a higher sensitivity to estradiol within behavioral areas in the central nervous system, an event demonstrated in female rats (Bridges and Byrnes 2006), might support the earlier achievement of estrus. Both hypotheses for the earlier estrus achievement in NUL goats deserve further investigation. The difference in the interval to estrus onset, comparing MULT with NUL, suggests a possible need to adapt, according to parity, the use of a flexible-timed artificial insemination approach (FxTAI; Carvalho-De-Paula et al. 2020), which is based on observation of estrus onset.

Regarding the interval from estrus to ovulation, MULT does ovulated earlier than NUL, but the total interval between sponge removal to ovulation was similar between MULT and NUL. Thus, for fixed-time AI protocols, it seems not to be necessary to adapt schedule according to parity, similarly to the proposed in Saanen goats (Fonseca et al. 2017). Estradiol elicits the preovulatory surges of GnRH, and LH, which promote ovulation. The earlier ovulation after estrus onset observed in MULT could had

Table 2 Reproductive parameters (mean ± S.E.M. or %) of cyclic nulliparous (NUL) and multiparous (MULT) Alpine, Nubian, and Saanen goats subjected to 6-day progestogen and cloprostenol-based estrus synchronization protocol, regardless of the administration or not of 200 IU equine chorionic gonadotropin (eCG) im administered 24 h before device removal

End points	Alpine		Nubian		Saanen		Total	
	NUL	MULT	NUL	MULT	NUL	MULT	NUL	MULT
Estrus response (%)	80.0 (8/10)	80.0 (8/10)	80.0 (8/10)	90.0 (9/10)	50.0 (5/10)	100.0 (6/6)	70.0 (21/30)	88.5 (23/26)
IREO (h)*	38.5 ± 4.0[a]	61.0 ± 4.4[b]	36.0 ± 3.5	34.7 ± 3.0	38.4 ± 1.7	56.0 ± 7.4	37.5 ± 1.9[a]	49.4 ± 3.5[b]
IEOV (h)**	33.5 ± 3.8[a]	19.5 ± 4.2[b]	27.0 ± 2.7	28.0 ± 2.7	24.5 ± 2.4	25.2 ± 2.7	29.1 ± 1.8	24.3 ± 2.1
IROV (h)***	66.0 ± 2.0	70.8 ± 3.3	70.0 ± 2.7	69.7 ± 2.5	62.8 ± 5.1	72.0 ± 7.7	66.7 ± 1.8	70.6 ± 2.2
Ovulation rate (%)	100.0 (10/10)	100.0 (10/10)	80.0 (8/10)	90.0 (9/10)	50.0 (5/10)	66.7 (4/6)	76.6 (23/30)	88.5 (23/26)
DOF (mm)****	7.5 ± 0.3	7.1 ± 0.2	6.6 ± 0.2	6.5 ± 0.2	6.3 ± 0.2	6.7 ± 0.2	6.9 ± 0.2	6.8 ± 0.1
Number of ovulations	1.3 ± 0.2[a]	2.2 ± 0.3[b]	1.1 ± 0.1	1.4 ± 0.2	2.2 ± 0.1	2.0 ± 0.3	1.4 ± 0.1	1.9 ± 0.2

[a, b] Differ within rows between the parity orders (NUL vs. MULT) within the same breed ($P < 0.05$)

( )Number/proportion of animals

* IREO, interval from sponge removal to estrus onset; ** IEOV, interval from estrus onset to ovulation; *** IROV, interval from sponge removal to ovulation; **** DOF, diameter of ovulatory follicle

occurred by a possibly more efficient response in the cascade that starts with estradiol and involves sequential stimulation of GnRH neurons, gonadotrophs, and follicular cells. Interestingly, we showed in here that eCG had also an effect in anticipating ovulation in MULT but not in NUL. This event supports the inference that the sensitivity of ovarian cells to eCG (an LH-like molecule) is higher in MULT, which also explain why they ovulate earlier after estrus onset, when compared to NUL. In Alpine does specifically, considering the higher number of ovulations in MULT, the ovulation threshold could be earlier achieved after estrus onset as a consequence of an additive estrogen production resulted by the greater number of eCG-responsible ovulatory follicles in this parity group. Considering the number of ovulations as a predictor of potential number of kids born, the significantly higher number of ovulations in MULT in comparison to NUL Alpine goats corroborated the greater prolificacy (1.9 vs. 1.4 kids born) previously reported in the former (Fonseca et al. 2005).

The similar estrus response and ovulation rate between NUL and MULT of all three breeds suggest a similar reproductive efficiency in does from both parity orders, corroborating with a previous study (Alvarado-Espino et al. 2019) using injectable progesterone and hCG. In fact, the present findings showed no difference between parity order for any reproductive parameter in G-Control. These results suggest that similar reproductive parameters may be achieved in either NUL or MULT goats in the absence of eCG. In addition, these data highlight that the G-eCG protocol can be recommended for providing great estrous response and ovulation synchrony, able to support both FxTAI (Carvalho-De-Paula et al. 2020) or FTAI (Fonseca et al. 2017), regardless of the dairy goat breed.

Conclusion

Although the short-term hormonal protocol of estrus synchronization without the administration of eCG was able to provide estrous response similar to that obtained by traditional protocols involving this hormone, the absence of eCG caused a decrease in the number of animals ovulating. Therefore, removing eCG from FTAI protocols should be parsimoniously considered. Future investigations are necessary to design preferable protocols. Furthermore, results of the present study suggest that both NUL and MULT goats may respond similarly to hormonal estrus synchronization stimuli during the breeding season.

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Code availability Not applicable.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval This study was approved by the Ethics Committee on Animal Use of Universidade Federal Fluminense (protocol #6405230719), and thus, it was conducted in line with all ethical standards required.

Consent to participate Not applicable.

Consent for publication All the authors consent to publish the manuscript.

Conflict of interest The authors declare no competing interests.

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