

Short Communication

Ovarian response to oestrous synchronization protocol based on use of reduced doses of cloprostenol in cyclic goats

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Contents

The objective of this study was to assess the efficacy of two reduced doses vs a high/luteolytic dose of cloprostenol on luteolytic activity and synchronization of oestrus in cyclic goats. Experiment 1, included 24 goats randomly allocated to three groups: control group (group H) received a single high dose of cloprostenol (87.5 µg; 1.0 ml; i.m.) and M and L groups, which received half (43.75 µg; 0.5 ml) and a third (26.25 µg; 0.3 ml) of the highest dose, respectively. Experiment 2, included 24 goats randomly assigned to the same experimental groups. Each group was treated using two injections of cloprostenol administered 10 days apart to synchronize oestrus. Transrectal ultrasonographic scanning (US) was performed to detect the presence, size and development of corpora lutea and ovarian follicles. Furthermore, detection of oestrus was performed every 12 h between 24 and 72 h after the second injection of cloprostenol, and the luteolytic effect was verified by US. In Experiment 1, all goats that had corpora lutea at timing of treatment regressed their corpora lutea. In Experiment 2, the occurrence of oestrus and the interval between treatment to onset of oestrus were: 100%, 49.5 ± 3.0 h; 100%, 51.0 ± 3.0 h; and 75%, 56.0 ± 3.5 h for H, M and L groups, respectively. The development of preovulatory follicles and occurrence of subsequent corpora lutea were similar among groups. In summary, the use of 26.25 µg of cloprostenol is effective for the synchronization of oestrus in cyclic goats.

Introduction

Prostaglandin F_{2α} and/or their analogues (PGF) have been used commonly to synchronize oestrus in goats during the breeding season (Thimonier 1981). Treatment with prostaglandins induces luteolysis and a subsequent follicular phase culminating in oestrus and ovulation (Greyling 1996). In does, the use of PGF analogues for oestrous synchronization consists of two PGF injections given 9–13 days apart (Ahmed et al. 1998; Greyling 1996). The response to PGF is efficient for oestrous synchronization (Greyling 1996), but fertility rate is reduced compared to use of progestagen sponges (80% vs 67%; Kusina et al. 2000). This reduction in fertility could be attributed to stages of luteal and follicular development at injection (Gonzalez-Bulnes et al. 2005), route of administration (i.e., intramuscular or intravulvo-submucosal routes), and broad range of doses administered (5–10 mg of dinoprost or 62.5–125 µg of cloprostenol; Mgongo 1987; Mellado et al. 1994).

In this regard, earlier studies performed in does (Moore and Eppleston 1979; Mgongo 1987) demonstrated that reduced doses of cloprostenol (i.e., 62.5 µg)

have been effective to induce luteolysis, oestrus, and normal fertility when compared with a conventional dose (125 µg). Additional studies are needed to evaluate the effectiveness of reduced doses of cloprostenol when administered as an intramuscular injection to cyclic goats.

Therefore, the objective of this study was to compare the ovarian response to two reduced doses of cloprostenol vs a high and effective dose in cyclic goats, in terms of luteolytic activity, follicle development, synchronization of oestrus, and subsequent corpora lutea (CL) generated.

Materials and methods

Animals

All experiments were carried out between May and July using 48 Canarias crossbred goats (aged from 2.7 to 8.7 years; 44.2 ± 0.9 kg). Goats were managed under natural conditions (day length and temperature) at the Unidad Experimental de Producción Caprina, located in Maracay (10°27'N), Aragua State, Venezuela. Goats were allocated in pens of 144 m² and fed 400 g of concentrate (18% crude protein) with free access to hay (*Cynodon dactylon*), minerals and water. The reproductive activity in does was previously confirmed weekly by detecting the presence of luteal tissue using transrectal ultrasonographic scanning (US).

Experimental design

Experiment 1: assessment of luteolytic activity of reduced doses of cloprostenol

Twenty-four does were selected to evaluate luteolytic activity of two reduced doses of cloprostenol (Planate^{®1}; 87.5 µg/ml) vs a high luteolytic dose. Goats were allocated randomly to three dose groups (n = 8 per dose). The first group ('High' group) was treated with 87.5 µg of cloprostenol (1.0 ml) using the intramuscular route (i.m.; Plumb 2008); the second and third groups ('Medium' and 'Low' groups) were treated with half (43.75 µg; 0.5 ml) or one third (26.25 µg; 0.3 ml) of the high dose of cloprostenol, respectively. Body weights were similar for the three treatment groups (high: 44.5 ± 2.2, medium: 45.2 ± 2.4 and low: 44.0 ± 2.7 kg; p = 0.9). To evaluate the luteolytic activity, US was used to confirm the presence, number and size of CL, on Days 0 (day of treatment), 3 and 7.

Experiment 2: assessment of follicular development, oestrus and corpora lutea

To evaluate the effect of reduced doses to synchronize the oestrus, a second group of 24 cyclic goats was assigned to the same treatment groups used in the Experiment 1 ($n = 8/\text{group}$): high (87.5 μg), medium (43.75 μg) and low dose (26.25 μg) of cloprostenol. Likewise, body weight was not different among treatment groups (high: 44.2 ± 2.2 , medium: 44.0 ± 2.4 and low: 43.4 ± 2.0 kg; $p = 1.0$). Each group was treated using two injections of cloprostenol given 10 days apart. The luteolytic response and presence of new CL generated were evaluated using US on Days 0 (day of first injection), 3, 7, 10 (day of second injection) and 11 days after of second injection. Scanning of preovulatory follicles was carried out on Days 10, 11, 12, and 13 to assess follicular development during the induced follicular phase. Simultaneously, oestrous detection was performed twice daily from 24 to 72 h after second injection, using vasectomized bucks.

Ultrasonographic scanning

Development of ovarian structures (CL and follicles) was assessed using a scanner (Aloka[®], SSD 500, Aloka Co., LtdJapan), fitted with a transrectal linear probe (7.5 MHz). Follicles were identified as anechoic and spherical structures, and CL were recognized as distinctive, homogeneous, and hypoechoic structures compared with the ovarian stroma. Follicle diameter (mm) and CL area (mm^2) were measured using the scanner electronic callipers. Size of CL was expressed as total area of luteal tissue, in those goats with two or more corpora lutea, and per CL. Area of the central cavity for a CL was measured and subtracted from the total CL area to calculate area of luteal tissue (Simões et al. 2005).

Statistical analyses

Differences among groups in terms of size of luteal tissue per goat, number and size per CL per goat, follicular growth (during follicular phase), and interval treatment to onset of oestrus were analysed using one-way ANOVA. All results were expressed as means \pm standard error and statistical differences were detected using the Tukey test. Number of goats showing luteolysis and oestrus was analysed using Fisher test. All analyses were performed using an analytical software (Statistix[®] Version 8.0).

Results

Assessment of the luteolytic activity and CL development

In Experiment 1, four does (two belonging to Medium group and one belonging to High and Low groups, respectively) were eliminated from the statistical analysis, due to lack of CL at the beginning of the experiment. However, the size and number of CLs were similar among groups on Day 0 (Table 1). Scanning performed on Day 3 after treatment did not detect presence of luteal tissue in treated does. Also, the number and size of CLs were similar among groups at Day 7 after the injection (Table 1).

Table 1. Experiment 1: size and number of corpora lutea (CL) on Days 0 (day of injection) and 7 in crossbreed Canarias does treated with high (87.5 μg), medium (43.75 μg) and low (26.25 μg) doses of cloprostenol

	Groups (n)		
	High (7)	Medium (6)	Low (7)
Day 0			
Area/goat (mm^2)	178.3 ± 33.1	210.2 ± 35.7	178.4 ± 33.1
Area/CL (mm^2)	124.8 ± 13.4	114.6 ± 12.8	96.1 ± 11.8
N° of corpora lutea	1.4 ± 0.2	1.8 ± 0.2	1.9 ± 0.2
Day 7			
Area/goat (mm^2)	93.0 ± 16.1	87.0 ± 17.4	90.8 ± 17.4
Area/CL (mm^2)	59.2 ± 5.1	52.2 ± 5.3	68.1 ± 5.9
No. of corpora lutea	1.6 ± 0.2	1.7 ± 0.3	1.3 ± 0.3

In Experiment 2, six does (2, 3, and 1 from high, medium and low groups, respectively) did not show ultrasonographic images compatible with luteal tissue on Day 0 (first injection). However, the number and size of luteal structures and CL from the remainder goats were similar among groups (1.7 ± 0.3 , 1.4 ± 0.3 and 1.3 ± 0.2 CLs/goat; 91.2 ± 19.3 , 115.4 ± 21.2 and 100.6 ± 17.9 mm^2 of luteal tissue/goat; 54.7 ± 10.0 , 82.4 ± 11.9 and 78.2 ± 10.5 mm^2/CL ; for groups high [$n = 6$], medium [$n = 5$] and low [$n = 7$], respectively). Luteolytic response was confirmed 3 days later, when all treated does (7/7) with the low dose, and 83.3% (5/6) and 80% (4/5; $p = 0.5$) of treated goats in High and Medium groups, respectively, had no CL images. In addition, the scanning demonstrated the presence of a corpus haemorrhagicum on Day 7, in those goats (68.6 ± 6.4 mm^2 per CL; $n = 6$) that did not show a CL at onset of treatment.

Seven days later after the first injection, all goats had similar total luteal tissue areas (124.3 ± 17.6 , 87.1 ± 17.6 and 108.1 ± 18.8 mm^2 ; for High, Medium and Low doses, respectively) and number of CLs (1.5 ± 0.2 , 1.5 ± 0.2 and 1.4 ± 0.2 , for high, medium and low doses, respectively). However, the size per CL was higher for high than the medium group (82.8 ± 5.2 and 58.1 ± 5.3 mm^2/CL ; $p < 0.01$) whereas does treated with the low dose had an intermediate value (75.7 ± 5.7 mm^2/CL).

Oestrous synchronization

On day of the second injection (Day 10), all goats had similar size and number of CLs. All does in high and medium groups showed oestrous behaviour, but 75% (6/8) in low group ($p = 0.3$). The interval from treatment to onset of oestrus was similar among groups (Table 2). Furthermore, 62.5% of the treated goats from high group showed oestrus between 36 and 48 h, while 50.0 and 66.7% of does from medium and low groups, respectively, started the oestrous behaviour between 48 and 60 h.

Follicular development during induced follicular phase was not different in terms of follicular diameter on Day of the second injection and 2 days later (onset of oestrus; Fig. 1a). Also, the growth rate of preovulatory follicles relative to day of oestrus was similar among groups (0.7 ± 0.3 , 1.2 ± 0.3 and 1.6 ± 0.4

Table 2. Experiment 2: percentage of oestrous, treatment-oestrous interval, luteal size and ovulation rate generated (at Day 11) in crossbreed Canarias does, synchronized using high (87.5 µg), medium (43.75 µg) and low (26.25 µg) doses of cloprostenol

	Groups (n)		
	High (8)	Medium (8)	Low (8)
Percentage in oestrous	100.0 (8/8)	100.0 (8/8)	75.0 (6/8)
Treatment from treatment to onset of oestrous interval (h)	49.5 ± 3.0	51.0 ± 3.0	56.0 ± 3.5
Luteal status at Day 11			
Number of ovulation	2.1 ± 0.3	1.9 ± 0.3	2.0 ± 0.3
Area/CL (mm ²)	101.3 ± 6.8 ^{ab}	119.0 ± 7.3 ^a	88.2 ± 8.4 ^b
Area/goat (mm ²)	215.3 ± 34.0	223.1 ± 29.8	204.5 ± 34.4

Group with different superscripts within rows are significantly different ($p < 0.05$).

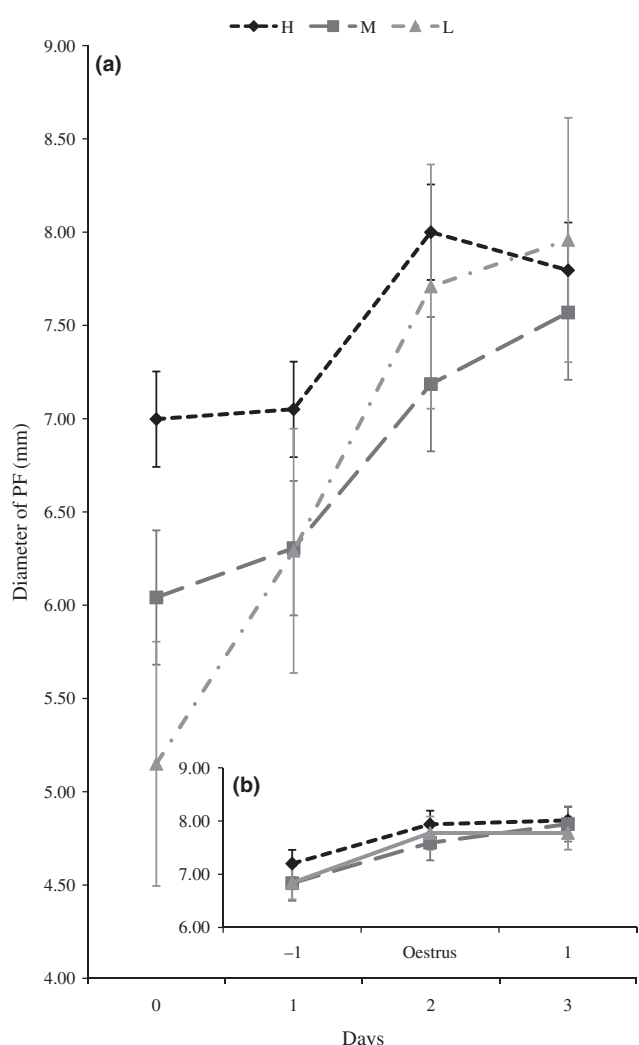


Fig. 1. (a) Growth of preovulatory follicles (PF) during the induced follicular phase in does treated synchronized with high ($H = 87.5$ µg), medium ($M = 43.75$ µg) and low ($L = 26.25$ µg) doses of cloprostenol. (b) Growth of preovulatory follicles relative to onset of oestrus.

mm/day; for high, medium and low doses, respectively; Fig. 1b).

Finally, ovulation rate and size of regressing luteal tissue per goat were similar among treatment groups on Day 11, 1 day after second injection of PGF. However,

the area per CL was different between does treated with medium and low doses ($p < 0.05$; Table 2).

Discussion

Results demonstrated that the use of a third (26.25 µg) of the standard dose of cloprostenol was effective to induce luteolysis. This effect was confirmed by the absence of luteal tissue on Day 3 after the injection, confirming that the effect and/or mechanism of action obtained in the present study is similar to those reported by Mgongo (1987) in goats, who observed a decrease in progesterone levels after 24 h, using luteolytic doses (125 and 62.5 µg) of cloprostenol given i.m.

When the scanning was performed just prior to the first injection (Experiment 2), six does did not show luteal tissue, suggesting presence of a follicular phase in these females. This was confirmed 3, 7, and 10 days later by US, when these animals had a numerically higher size of luteal tissue than those goats that had CLs on day of the first injection. In other words, those goats with luteal tissue and those without luteal tissue at the time of first injection developed CLs that were between 7–8 and 10–11 days of age, respectively at the day of the second PGF injection.

Regarding the percentage of does expressing oestrous behaviour, all doses were effective to induce oestrus; although the percentage response was numerically less in the low dose PGF group. This latter response was not statistically significant and would require many more does per treatment group to determine if this is indeed a biological difference attributed to a low dose of PGF. Also, the interval from PGF injection to the onset of oestrus was similar to those obtained in goats treated with 62.5, 100 and 125 µg of cloprostenol (Ahmed et al. 1998; Gonzalez-Bulnes et al. 2005). In addition, all treated does showed similar follicular development in terms of size (diameter) and growth rate during the induced follicular phase. Nevertheless, the diameter of preovulatory follicles at the time of second injection was numerically larger for high compared with medium and low doses. This may explain why more than 60% of does treated with high doses had an earlier onset of oestrus (36–48 h) than does treated with medium and low doses (48–60 h). These results are supported by Gonzalez-Bulnes et al. (2005), who reported a positive correlation between follicular status/size and onset of oestrus.

On the other hand, despite of the luteolytic activity observed in all does after the second injection, two does treated with the Low dose did not show oestrus. This could be due to anovulatory follicles detected by US. Baird (1983) indicated that luteinizing hormone (LH) is essential for the final maturation of the preovulatory follicle. Therefore, the presence of anovulatory follicles could be caused by an insufficient preovulatory LH discharge (Fatet et al. 2010). Additionally, the absence of normal follicular development in two goats might be attributed to other factors (e.g. individual variability), besides the treatment, because the low dose induced luteolysis.

In summary, present results demonstrated that the use of 26.25 µg of cloprostenol given i.m. route was effective to induce luteolysis, oestrous behaviour, and to generate viable CL during the subsequent cycle. Thus, its use

could be included in oestrous synchronization protocols in cyclic goats.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

CD and ICS: Experimental phase and data analyses, manuscript editing. AS and TD: manuscript editing.

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