

Effects of prostaglandin administration 10 days apart on reproductive parameters of cyclic dairy nulliparous goats

[Efeitos da aplicação de prostaglandinas intervaladas de 10 dias sobre características reprodutivas de cabras leiteiras núlparas cíclicas]

J.F. Fonseca¹, V.V. Maffili², A.D.F. Santos², R. Fürst², C.P. Prospero², H. Rovay²,
J.M.G. Souza², C.A.A. Torres²

¹Embrapa Caprinos e Ovinos - Coronel Pacheco, MG

²Universidade Federal de Viçosa - Viçosa, MG

ABSTRACT

This study reported the effects of prostaglandin (PGF_{2α}) administration 10 days apart on reproductive parameters of cyclic artificial inseminated (AI) nulliparous Alpine (n=9) and Saanen (n=9) goats. Animals received two doses of 22.5µg PGF_{2α} 10 days apart. After 1st and 2nd-PGF_{2α} administrations, estrus was monitored at 12 h intervals, with a buck teaser. Plasma progesterone concentration (ng/mL) was determined from blood sampled on day 0 (1st-PGF_{2α}) and the following 5, 10 (2nd-PGF_{2α}), 15, 20, 25 and 30 days. After the onset of the second estrus, females were transrectally (5 MHz probe) scanned at 4 hour intervals until at least 8h after ovulation. Pregnancy was checked through transrectal ultrasound on days 20, 25, 30, 35 and 90 after insemination. All parameters studied did not differ between breeds (P>0.05). Estrous response and interval to estrus, respectively, after 1st (78.9% and 50.6±17.2h) and 2nd PGF_{2α} (88.9% and 50.0±14.8h) administration did not differ (P>0.05). Overall animals ovulating (100.0%), interval to ovulation after 2nd-PGF_{2α} (64.5±19.5h) and after estrus onset (18.0±9.1h), ovulation rate (1.3±0.5), diameter of ovulatory follicle (8.1±1.1mm) were recorded. Embryo loss occurred before day 30 of pregnancy. Estrus can be efficiently synchronized in nulliparous Alpine and Saanen goats with two doses of prostaglandin 10 days apart.

Keywords: estrus, ovulation, progesterone, embryo loss, goat

RESUMO

Relataram-se os efeitos da aplicação de prostaglandina sobre características reprodutivas de cabras leiteiras núlparas cíclicas. Cabras Alpinas (n=9) e Saanen (n=9) receberam duas doses de 22,5µg PGF_{2α} com 10 dias de intervalo. A progesterona plasmática (ng/mL) foi determinada a partir de amostras de sangue coletadas nos dias 0 (primeira dose), 5, 10 (segunda dose), 15, 20, 25 e 30. Após início do segundo estro, as fêmeas foram monitoradas por ultrassonografia transretal a cada quatro horas até oito horas após a ovulação. A gestação foi verificada por ultrassonografia transretal nos dias 20, 25, 30, 35 e 90 após a segunda dose. As características estudadas foram semelhantes entre as raças (P>0,05). Animais em estro e o intervalo parto-estro de, respectivamente, 78,9% e 50,6±17,2h e 88,9% e 50,0±14,8h após a primeira e segunda administrações de prostaglandina, não diferiram (P>0,05). Todas as cabras ovularam e registraram-se valores do intervalo parto-ovulação após a segunda aplicação de prostaglandina de 64,5±19,5h e após início do estro de 18,0±9,1h, a taxa de ovulação de 1,3±0,5 e diâmetro do folículo ovulatório de 8,1±1,1mm. Perda embrionária ocorreu antes de 30 dias de gestação. O estro pode ser eficientemente sincronizado em cabras leiteiras núlparas com duas doses de prostaglandina intervaladas de 10 dias.

Palavras-chave: caprino, estro, ovulação, progesterona, perda embrionária

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E-mail: jeferson@cnpq.embrapa.br

INTRODUCTION

The concentration of herd reproductive activities has a direct impact on breeding management and handling. In this context, estrous synchronization can be a valuable tool, since it allows at any given week day activities such as artificial insemination and embryo transfer, easier estrous identification which facilitates reproductive management as well as allows birth synchronization and thus meat and milk production.

Basically there are three ways to synchronize estrus in goats. First, in anestrus goats by using a combination of progestagens, gonadotrophins and prostaglandins through light programs or melatonin implants. Second, by male effect in females previously isolated (one to three months) from the buck's presence, which may also activate the estrous synchronization cycle as well as anticipate the reproductive activity in anestrus females (transition season). The third form includes prostaglandin administration either in a single dose or in two doses 10 to 11 days apart during breeding season (Fonseca *et al.*, 2007).

An important factor to consider in estrous synchronization and superovulation is the moment of ovulation. Its recognition and association with the interval elapsed from the administration of prostaglandin and onset and duration of estrus may generate more efficient protocols of artificial insemination, based on estrous detection and at a fixed time. The moment of ovulation was initially recognized by laparoscopy (Chemineau, 1983) and nowadays may be easily identified by ultrasound (Castro *et al.*, 1999). Soon one may monitor ovarian dynamics from the onset of estrus and identify ovulatory follicles and its rupture, and formation of the hemorrhagic body, based on each structure ecographic characteristics. Additionally, the recognition of possible differences between breeds, as well as hormonal profiles during and after synchronization may be very important.

The objective of this study was to evaluate the effects of administering two doses of prostaglandin at 10 days apart on reproductive parameters of nulliparous Alpine and Saanen goats.

MATERIALS AND METHODS

This study was conducted during the months of May and June in a location with the following coordinates: 20°45'S latitude and 42°51'W longitude; altitude of 692.73m with CWA (dry winter and humid summer) climate, annual average temperature of 20.9°C and annual rainfall of 1,203mm³. At these coordinates, the transitional portion of the seasonal breeding pattern extends from December to February, the breeding season from March (end of summer) to June (end of autumn) and the anestrus season from July to November.

The experiment used Saanen and Alpine breeds. Nine Alpine (aged 8-18 months and weighting 31.3±1.9kg, and nine Saanen, aged 8-18 months and weighting of 37.5±7.7kg goats were used. Body condition score (scale 1 to 5) was assessed and showed 2.5±0.2 e 3.0±0.7, respectively, for Alpine and Saanen goats. Goats were kept on suspended pens and fed *ad libitum* with corn silage and concentrated ration twice a day according to nutrient demand. Water and mineral salt were permanently available.

Animals received two doses of 22.5µg synthetic prostaglandin (d-cloprostenol; Prolise[®], ARSA S.R.L., Buenos Aires, Argentina) intra-vulvo submucosal at 10 days interval. After the first prostaglandin administration, estrus was monitored twice daily from 06:00 to 08:00AM and 4:00 to 06:00PM, with the aid of buck teasers. Estrous detection persisted for 10 days until the administration of the second dose of prostaglandin, when all animals were evaluated through transrectal ultrasound with the help of a 5MHz probe (Aloka[®], SSD – 500, Tokyo, Japan) coupled to polyethylene support. After the administration of the second dose of prostaglandin, doses were observed every four h in order to accurately identify the onset of estrus. Once estrus was identified, animals were submitted to ultrasound evaluation every four h until ovulation detection and at least for the next 8h. Ovulation was considered as having occurred when previous detected follicles collapsed and could not be observed (Ginther and Kot, 1994). Both ovary and uterus were monitored. After ovulation detection, females were inseminated with frozen-thawed semen (0.25mL straws/100x10⁶ spermatozoa) of proved fertility with a minimum of 50% of progressive

motility after thawing and 40% HOST reaction (Fonseca *et al.*, 2005). All females were evaluated through ultrasound following an elapsed period of 20, 25, 30, 35 and 90 days after the second prostaglandin administration, in order to prematurely detect and confirm pregnancy.

Blood was sampled on days 0 (first prostaglandin administration), 5, 10 (second prostaglandin administration), 15, 20, 25 and 30 through jugular puncture in heparinized vacuolated tubes. After collection, the tubes were kept on ice until centrifugation in a refrigerated centrifuge at 5°C, at 2500×g/15 min. Plasma was then aspired and stored at -20° C until analysis. Time from blood collection to plasma aspiration did not exceed 2h. Plasma progesterone concentration (ng/mL) was determined through the use of the solid phase radioimmunoassay, using a commercial kit (Coat-a-count® progesterone kit, DPC, Diagnostic Products Co., Los Angeles, CA, USA). The mean intra- and inter-assay coefficient of variation was 7% and 8%, respectively.

The following characteristics were determined: estrous response; interval to estrus (h): time from prostaglandin administration to the onset of estrus; interval to ovulation (h): time from second prostaglandin administration to ovulation; interval to ovulation after estrous onset (h): time from estrous onset to ovulation; interval to artificial insemination (AI; h): time from second

prostaglandin administration to AI; interval to AI after estrous onset (h): time from estrous onset after second prostaglandin administration to AI; percentage (%) of ovulating animals; ovulation rate; diameter of ovulatory follicle (mm): maximum diameter of follicle previously detected before ovulation; ovulation on left or right ovary (%); plasma progesterone concentration (ng/mL); number of fetuses per goat artificially inseminated; conception rate (%).

Statistical analysis included one way analysis of variance for testing the differences in variables studied between nulliparous Alpine and Saanen goats. Parametric variables were tested with the aid of Tukey test processed with SAEG. Non-parametric variables were analyzed using χ^2 procedures. Linear Pearson correlation was performed (Ribeiro Júnior, 2001). All tests were considered at minimum level of 5% significance.

RESULTS

The percentage of animals in estrus after the first and the second prostaglandin administrations did not differ between breeds (P>0.05; Table 1). Two responsive goats showing estrus 36 and 48h after first dose (one Alpine and one Saanen) displayed another estrus five days after this one. These animals did not present estrus after second prostaglandin administration.

Table 1. Estrous response (%) of Alpine and Saanen goats submitted to administration of two doses of cloprostenol 10 days apart

Breed	First prostaglandin	Second prostaglandin
Alpine	88.9% (8/9)	88.9% (8/9)
Saanen	66.7% (6/9)	88.9% (8/9)
Total	77.8% (14/18)	88.9% (16/18)

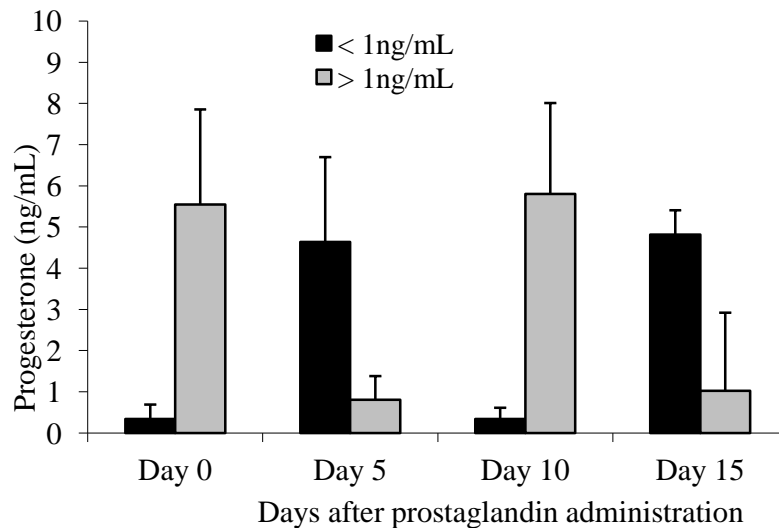
The interval to estrus and time to artificial insemination relative to these times are reported in Table 2. Negative correlation was observed between interval to estrus after first prostaglandin administration (r=-0.55; P<0.05) and interval to estrus after second prostaglandin administration.

Four goats showed P4 concentration inferior to 1ng/mL on day 0; three did not come into estrus and one came into estrus 14h after first cloprostenol administration. These four goats showed lower (P<0.05) P4 (0.3±0.3ng/mL) at first cloprostenol

administration when compared with those which came into estrus (5.6±2.3ng/mL). A similar pattern was observed for the second cloprostenol administration, when three goats with P4 lower than 1ng/mL did not come into estrus. These three goats showed lower (P<0.05) P4 (0.3±0.3ng/mL) at second cloprostenol administration when compared with those that came into estrus (5.8±2.2ng/mL)(Figure 1). On the other hand, goats that did not come into estrus after first or second cloprostenol administration showed higher (P<0.05) P4 on days 5 and 15 (4.6±2.1 and 4.8±0.6ng/mL) than those showing estrus (0.8±0.6 and 1.0±1.9ng/mL), respectively.

Table 2. Reproductive characteristics (hours) after first and second dose of prostaglandin (PGF_{2α}) administered 10 days apart in nulliparous Alpine and Saanen goats (mean ± SD)

Characteristic	Alpine	Saanen	Total
Interval to estrus after 1 st PGF _{2α}	49.5±20.2 (n=8)	52.0±14.2 (n=6)	50.6±17.2 (n=14)
Interval to estrus after 2 nd PGF _{2α}	49.5±16.8 (n=8)	50.5±13.8 (n=8)	50.0±14.8 (n=16)
Interval to ovulation after 2 nd PGF _{2α}	66.7±17.9 (n=8)	62.2±21.9 (n=8)	64.5±19.5 (n=16)
Interval to ovulation after estrous onset	19.2±8.6 (8)	16.7±10.0 (n=8)	18.0±9.1 (n=16)
Interval from estrous onset to AI	21.2±8.6 (8)	18.7±10.0 (8)	20.0±9.1 (n=16)
Interval from 2 nd PGF _{2α} to AI	68.7±17.9 (n=8)	64.2±21.9 (n=8)	66.5±19.5 (n=16)

Figure 1. Nulliparous dairy goats submitted to estrous synchronization with two doses of 22.5µg cloprostenol (PGF_{2α}) on days 0 and 10 that showed plasma progesterone concentration (ng/mL) lower or higher to 1ng/mL on the moment of the cloprostenol administration.

Ovulatory characteristic of estrous synchronized goats submitted to ultrasonography evaluation are shown in Table 3.

Negative correlation was observed between interval to estrus after the first prostaglandin administration and interval to ovulation after the second prostaglandin administration ($r=-0.57$; $P<0.05$). One Alpine goat was removed from the experiment during ultrasound evaluation because of accidental natural breeding.

In both breeds embryo loss occurred before ultrasonography on the 30th day. Ultrasonography performed on day 20 of the studied animals (17 to 18 days after ovulation)

was 94.7% efficient in order to detect pregnancy precociously (vesicles). Only one goat which showed higher progesterone (11.6ng/mL) on day 20 was not classified as pregnant through the same day ultrasound. However, this goat came into estrus five days later (day 25). One Alpine goat with 1.5ng/mL P4 on day 20 was pregnant at ultrasonography, but this goat came into estrus four days before day 20. For the other animals, those with progesterone under 0.5ng/mL (0.2 ± 0.1 ng/mL; range 0.1 to 0.4ng/mL) or higher than 1ng/mL (7.1 ± 0.5 ng/mL; range 2.8 to 8.9ng/mL) were classified as non-pregnant and pregnant, respectively, through ultrasonography on the 20th day.

Table 3. Ultrasonography characteristics of ovarian follicles after the second prostaglandin administration in nulliparous Alpine and Saanen goats submitted to two doses of prostaglandin 10 days apart

Parameter	Alpine	Saanen	Total
Number of animals	8	8	16
Ovulation in right ovary (%)	7(63.6%)	7(70.0%)	14(66.7%)
Ovulation in left ovary (%)	4(36.4%)	3(30.0%)	7(33.3%)
Goats that ovulated (%)	8(100.0%)	8(100.0%)	16(100.0%)
Ovulation rate	1.37±0.5	1.25±0.5	1.31±0.5
Diameter of ovulatory follicles (mm)	8.2±1.2	8.0±1.1	8.1±1.1

P4 did not differ ($P>0.05$) between Alpine and Saanen goats during days studied (Figure 2). Vesicles counts recorded 1.6 and 1.0 for Alpine and Saanen goats, respectively at day 20 after the second cloprostenol administration. From four Alpine goats with two vesicles at this time, two remained with both vesicles, one lost one vesicle, and one lost both vesicles. From seven goats with one vesicle, two Alpine and three Saanen goats lost their vesicles until the end of the study.

Goats were grouped as pregnant (vesicles present) and non-pregnant (vesicles absent)

at day 20 after the second prostaglandin administration. P4 was higher ($P<0.01$) in pregnant than in non-pregnant at this time (Figure 3).

Embryo detection and loss are presented in Figure 4. The number of pregnant animals detected via ultrasound 20 days after the administration of the second dose of prostaglandin (\cong day 18 after ovulation) was 7/8 (77.8%) in the Alpine and 5/8 (62.5%) in the Saanen breed.

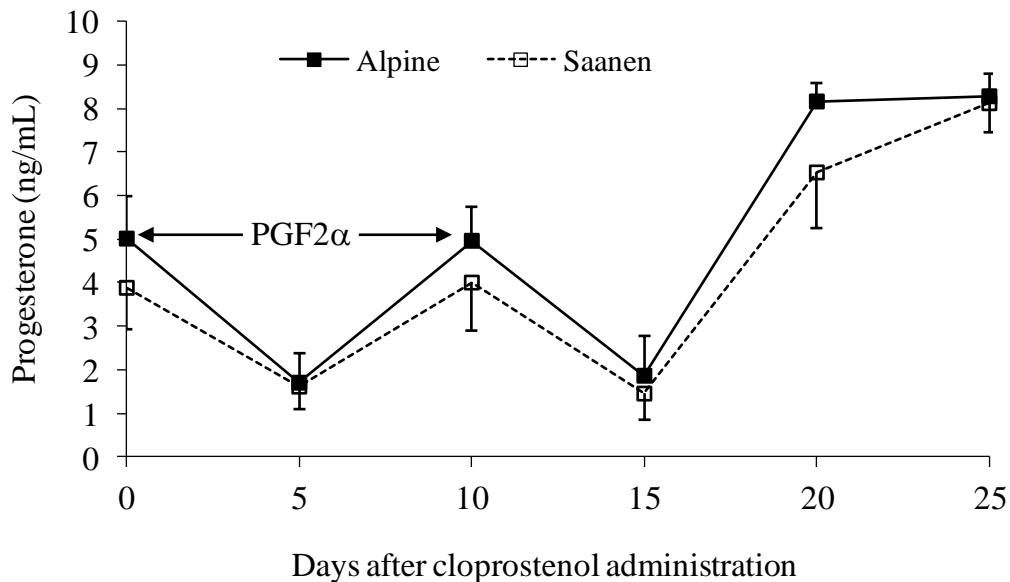


Figure 2. Plasma progesterone concentration (ng/mL) of nulliparous Alpine and Saanen goats submitted to estrous synchronization with two doses of 22.5 µg cloprostenol (PGF2α) 10 days apart.

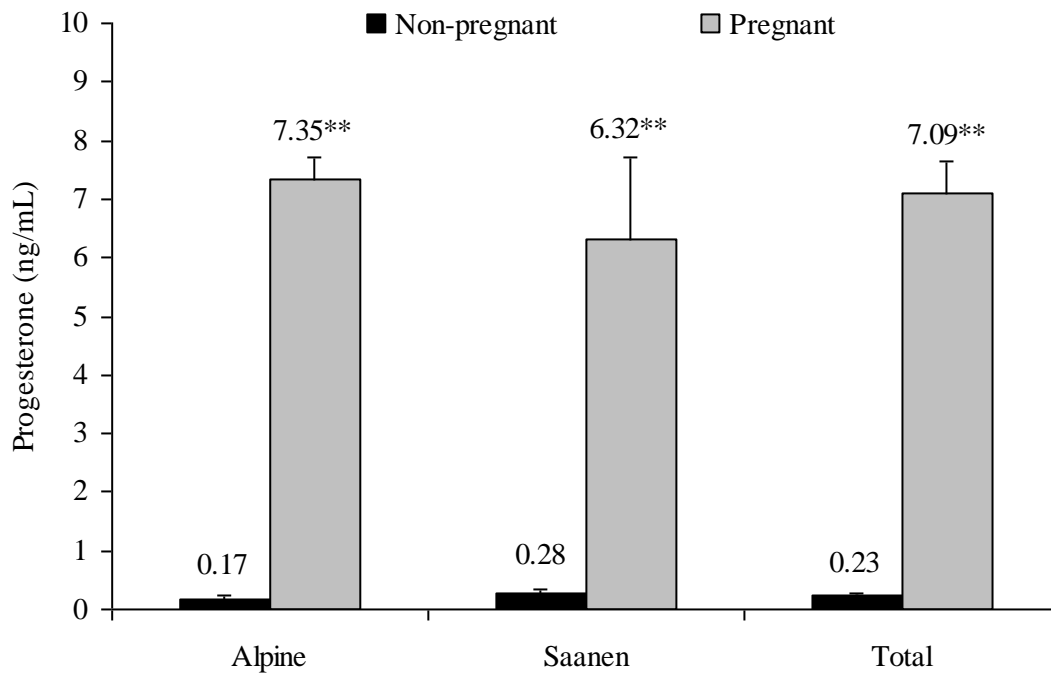


Figure 3. Plasma progesterone concentration (ng/mL) of artificial inseminated nulliparous Alpine and Saanen goats with apparent pregnant uterus detected by ultrasonography at day 20 after second cloprostenol administration. ** P<0.01.

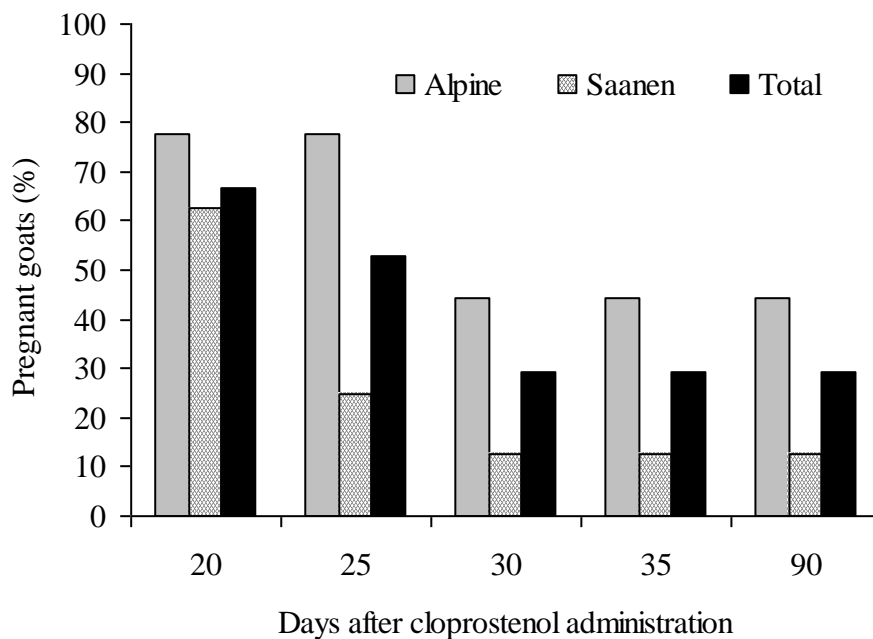


Figure 4. Pregnancy rate (%) of artificial inseminated nulliparous Alpine and Saanen goats checked via ultrasonography from days 20 to 90 after the second cloprostenol administration.

In both breeds embryo loss occurred before ultrasonography on the 30th day. Ultrasonography performed on day 20 of the studied animals (17 to 18 days after ovulation) was 94.7% efficient in order to detect pregnancy precociously (vesicles). Only one goat which showed higher progesterone (11.6ng/mL) on day 20 was not classified as pregnant through the same day ultrasound. However, this goat came into estrus five days later (day 25). One Alpine goat with 1.5ng/mL P4 on day 20 was pregnant at ultrasonography, but this goat came into estrus four days before day 20. For the other animals, those with progesterone under 0.5ng/mL (0.2 ± 0.1 ng/mL; range 0.1 to 0.4ng/mL) or higher than 1ng/mL (7.1 ± 0.5 ng/mL; range 2.8 to 8.9ng/mL) were classified as non-pregnant and pregnant, respectively, through ultrasonography on the 20th day.

As can be seen on Fig. 4, the ultrasonography revealed that embryonic losses occurred before the first 30 days of pregnancy. Under the conditions of this study, embryos/fetuses that survived up to this time were not lost until 90 days of pregnancy.

DISCUSSION

The percentage of animals in estrus after the first cloprostenol administration was similar to those related by Goel and Agrawal (1998), who reported 81.8% estrous response in goats without definite breed and 75.0% in Jakhrana lactating goats. At that moment, forming corpus luteum was not susceptible to prostaglandin effects. Thus, short estrous cycle can decrease the estrous response after the second prostaglandin administration.

Furthermore, the percentage of animals in estrus after the second administration of prostaglandin was similar to the one described by Pandey *et al.* (1985) in cyclic Black Bengal goats (86.0%). The same dose and intervals used in the present study were also studied by Siqueira *et al.* (2009) in Toggenburg goats who reported 100% estrous response after second prostaglandin administration. Previous studies reported dosages ranging from 50 to 250 μ g of the same drug (Baril *et al.*, 1993; Kusima *et al.*, 2000).

Overall, the interval to estrus observed in this study after first (50.1h) and second (50.0h)

prostaglandin administration was smaller than those (71.3 and 65.3h) reported by Goel and Agrawal (1998), respectively in Jakhrana and unknown origin goats. The smaller interval related in this study as compared to that could be a result of administration route. Mellado *et al.* (1994) reported a smaller interval from prostaglandin administration to the onset of estrus by comparing the use of intra-vulvo submucosal to the results of intramuscular route. Thus, it appears that the intra-vulvo submucosal route could allow a quicker access and a greater quantity of molecules to the target tissue, the corpus luteum, averting a first passage and hepatic metabolism. Siqueira *et al.* (2009) reported a 46h interval to estrus after second prostaglandin administration in Toggenburg goats using the same via. In Nubian goats submitted to two prostaglandin administrations 12 days apart, Romano (1998) reported interval to estrus higher after first (72h) and similar after the second prostaglandin administration (52h) than those reported in the present study.

Goat experiencing estrus later after first prostaglandin administration could probably be in growing follicular pattern and have follicles capable of ovulating if luteal progesterone was removed. This model was proposed by Menchaca and Rubianes (2004). Thus, according to interval between prostaglandins, one or two follicular waves can emerge in this period. If luteolysis is evoked during the follicular wave's final growing phase, animals come into estrus and ovulate earlier than those animals which luteolysis occurred at the initial rump of follicles growing.

As shown in Figure 1, the presence of an active corpus luteum at the time of the prostaglandin administration is the key point to determine the success of estrous synchronization. Animals with P4 shorter than 1ng/mL at this time could have been in estrus two or three days earlier, or entering in estrus at the moment of the prostaglandin administration. Consequently, at day five pos prostaglandin administration, these animals are expected to have higher P4 when compared to those that suffer luteolysis and came into estrus in response to prostaglandin.

Menchaca *et al.* (2007) working with cyclic pluriparous Alpine goats submitted to estrous

synchronization with short-term progesterone protocol (five days) without eCG administration reported 100% goats ovulating, 1.5 ovulation per goat and 9.9mm diameter of ovulatory follicles. Maffili *et al.* (2006), with protocol similar to a previous study but with eCG administered at sponge or CIDR removal, reported 100% goats ovulating, 1.5 ovulation per goat and 7.7mm diameter of ovulatory follicles in Toggenburg goats.

The higher ovulation frequency of the right ovary noted in this study corroborates the conclusion of Prasad *et al.* (1980). Camp *et al.* (1983) also noticed that the right ovary is more active than the left one. Chávez *et al.* (1987) noted this phenomenon in rats when they showed the asymmetry of the information carried between right and left vagal nerve to ovaries. The uni or bilateral section of the vagal nerve produced different results depending on the *in situ* ovary. Ovulation rate in rats unilaterally ovariectomized was smaller in the left ovary when compared to the right one (42 vs. 84%). The authors concluded that, in the model used, regulatory compensatory systems are more likely to occur in the right ovary than in the left one. On the other hand, Romano and Abella (1997) did not find activity differences between the right and left ovaries in Nubian goats.

Higher P4 in pregnant goats 30 days after the second administration of prostaglandin was expected because non-pregnant animals could have had or were close to having luteolysis, as shown by Regueiro *et al.* (1999). The presence of functional corpus luteum in the moment of prostaglandin administration is required to successfully synchronize estrus. This corpus luteum must be responsive to prostaglandin activity, which happens on the fourth day of the estrous cycle in goats (Ott *et al.*, 1980).

Pregnant animals detected via ultrasound 20 days after the administration of the second dose of prostaglandin (\cong day 17 to 18 after insemination) were confirmed by their P4. This proves the strong association between ultrasound and hormonal diagnostic exams, as noted by Engeland *et al.* (1997) when they studied 50 day pregnant goats. Using 5 MHz transrectal probe, Martinez *et al.* (1998) also commented on the presence of small vesicles in non-pregnant animal's uterine lumen. They also reported that

the first evidences of the presence of concepts were detected on day 15 and cardiac pulse on day 19. The present study confirms the possibility of precocious identification of goat pregnancy. The possibility of hormonal dosage strengthens this diagnosis.

It was previously reported that although confirmed pregnant and giving birth according to first estrus, an Alpine goat showed a second estrus 15 days after the first one (Fonseca *et al.*, 2006). Thus this kind of finding appears to be repetitive for goats.

The results of this study confirm the possibility of precocious identification of goat pregnancy (<20 days). However, given embryonic mortality, it is suggested that a later evaluation (after 30 days) should be made to confirm pregnancy. Thus, there is no practical use for earlier detection of pregnancy in goats.

Finally, the central objectives of this study were to test the efficiency of estrous synchronization with prostaglandins and the identification of the ovulation moment. Due to this, goats were inseminated after ovulation, and the quality and viability of the formed embryo could be compromised. In cattle, Saacke *et al.* (2000) noted that insemination at the moment of estrous detection lead to better quality embryos but with a smaller fertilization rate. Inseminations performed 24h after the onset of estrus resulted in higher fertilization rates but also increased the amount of embryos with inferior quality and degenerated ones. Inseminations performed 12h after the onset of estrus resulted in intermediary results. Additionally, Motlomelo *et al.* (2002) observed a 52% pregnancy rate in progesterone induced estrous goats. They inferred that the probable explanation of the relatively small pregnancy rate was a consequence of insemination at the end of the estrus.

CONCLUSIONS

Estrus can be efficiently synchronized in Alpine and Saanen breeds using two doses of prostaglandin 10 days apart. The identification of the ovulation relative to the estrous onset is very important to establish effective protocols of insemination. Ultrasound supervision associated with plasma hormone (progesterone) determination is an important study tool.

Embryos may be visualized and their development may easily be followed. However, precocious diagnosis should be confirmed in more advanced pregnancy stages given the possibility of embryonic losses occurring after that.

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