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## Effect of high progesterone concentrations during the early luteal phase on the length of the ovulatory cycle of goats

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### Abstract

The effect of exogenous progesterone exposure early in the oestrous cycle on the duration of the interovulatory interval was studied in dairy goats. A controlled intravaginal drug release (CIDR-G) device was inserted for 5 days starting at day 0 (D0 group,  $n = 6$ ) or day 3 (D3 group,  $n = 5$ ) postovulation. A third group was composed of untreated control goats (control group,  $n = 7$ ). Daily transrectal ultrasound was carried out during the interovulatory interval to assess the ovarian dynamics. Oestrous behaviour was checked twice a day and serum progesterone levels were assayed in daily jugular blood samples. Treated goats showed two different responses. In three D0 goats and one D3 goat, progesterone concentrations fell immediately after CIDR withdrawal and this was followed by oestrus and ovulation between days 8 and 11 (short cycles). In the other three D0 goats and in four D3 goats the treatment significantly reduced the interovulatory interval ( $18.3 \pm 0.3$  and  $18.5 \pm 0.3$  days, respectively) (shortened cycles) compared with the control group ( $20.0 \pm 0.2$  days;  $P < 0.05$ ), but the intervals with progesterone concentrations over 1 ng/ml were not different ( $15.7 \pm 0.3$ ,  $15.8 \pm 0.7$  and  $16.0 \pm 0.5$  days for D0, D3 and control goats, respectively). In all D0 goats with a short cycle response, the ovulatory follicle arose from the first follicular wave but in the D3 goat with a short cycle it arose from the second follicular wave. These results showed that premature progesterone exposure early in the ovulatory cycle of the goat affected its length inducing short or shortened cycles. The effect of progesterone could either affect luteotropic support of the corpus luteum (CL) and/or stimulate a premature release of the luteolysin. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Ultrasonography; Luteolysis; Progesterone; Estrous cycle; Goat

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## 1. Introduction

Progesterone/progestogen treatments have been widely used to synchronise oestrus in goats and sheep, traditionally by the use of intravaginal devices for long periods (12–14 days). However, recent studies showed that a progesterone priming as short as 5–6 days is as effective as the traditional long-term primings to induce oestrus with acceptable pregnancy rates during seasonal anestrus in goats and sheep (Rubianes et al., 1998; Ungerfeld and Rubianes, 1999). In sheep, despite the better conception rate obtained, the efficacy of the short progesterone priming for oestrous synchronisation during the breeding season remains limited due to the presence of a still functional corpus luteum (CL) in some of the ewes (Viñoles et al., 2001). However, with short-term progesterone primings we obtained an unexpected high percentage of synchronised oestrus in cycling dairy goats (Rubianes et al., 1999).

In ewes with shorter oestrous cycles higher progesterone levels were found between days 2 and 4 post-oestrus than in ewes with longer cycles (Nephew et al., 1991). Previous studies have demonstrated that daily progesterone administration for a few days early after oestrus shortens the oestrous cycle in ewes (Woody et al., 1967) and cattle (Ginther, 1970). In addition Burke et al. (1994) found that in heifers the elevation of progesterone concentrations from days 1 to 5 but not from days 4 to 9 after oestrus reduced the lifespan of the CL and produced some “short” (8 days) but mainly “shortened” (18 days) cycles compared to controls heifers (21.2 days). However, similar studies in goats have not been conducted yet.

Because of our previous observation in goats and the results observed in sheep and cattle, we tested the hypothesis that high levels of progesterone administered early during the oestrous cycle shortens the inter-oestrous and interovulatory intervals of dairy goats.

## 2. Materials and methods

Multiparous non-lactating Saanen/Anglo Nubian cross-breed dairy goats were used during the breeding season (May–June) at the experimental laboratory of the Department of Physiology, Montevideo, Uruguay (35° SL). The does weighed  $45.6 \pm 3.0$  kg (mean  $\pm$  S.E.M.), had a mean body condition score of  $2.9 \pm 0.1$  (scale: 0–5) were fed alfalfa hay and pellets (mean: 1500 and 700 g per goat per day, respectively), and water was available ad libitum. The goats were housed outdoors in a sheltered pen (30 m  $\times$  30 m), and indoor box stalls (3 m  $\times$  3 m) were used for ultrasonic examinations.

Oestrous cycles were synchronised with two injections of a PGF2a analogue (160  $\mu$ g, Glandinex, Universal Lab, Montevideo, Uruguay) 9 days apart. On the following spontaneous cycle, the goats were randomly assigned to a control group (Ctrl.  $n = 7$ ) and two treated groups ( $n = 6$  each).

In the treated groups progesterone was administered for 5 days using a controlled intravaginal drug release device (CIDR-G, Eazi Breed, InterAg, New Zealand) containing 0.3 g of progesterone, starting on day 0 (group D0) or day 3 (group D3) after the ovulation was detected by ultrasonography. Oestrous behaviour was checked twice a day for 45 min with

a vasectomised buck throughout the experimental period. Oestrus was defined as the time when the goat stood to be mounted by the buck. The interoestrous intervals were classified as “short” when its duration was shorter than 12 days or “normal” when it ranged between 17.5 and 22 days. Normal interoestrous intervals with a statistically shorter mean duration than control goats were defined as “shortened cycle”.

Ovarian images were obtained with a B-mode machine and a 5 MHz linear array transducer (Aloka 210, Tokyo, Japan). A slightly arched plastic open tube (diameter 2.5 cm; length 40 cm) was fastened to the transducer with duct tape so that the probe could be manipulated externally into the rectum (Rubianes et al., 1996). The procedure to locate the ovaries was the same as described by Ginther and Kot (1994). The diameter, positions and characteristics of the CL and the antral follicles  $\geq 3$  mm in diameter were recorded. Examinations were also recorded on videotape, one tape per goat (Sony, Tokyo, Japan), for further analysis of data. A follicular wave was defined as the emergence of a group of small follicles ( $\geq 3$  to  $< 4$  mm in diameter) that gave origin to 1 or more follicles  $\geq 5$  mm. The day of emergence of the wave was the day of emergence of the largest follicle of that wave, and more than 1 day was allowed for all the follicles of a wave to emerge. Ovulation (day 0) was detected by collapse of a large follicle.

An amount of 10 ml blood samples were taken daily by jugular venipuncture for progesterone determinations. Samples were allowed to clot at room temperature and were centrifuged within 2 h after collection. The serum was stored at  $-20^{\circ}\text{C}$  until assayed for progesterone. The progesterone concentrations were determined by a direct solid-phase RIA (DPC, Diagnostic Product Co., Los Angeles, CA, USA), previously described (Rubianes and Ungerfeld, 1993). The detection limit of the assay was 0.1 ng/ml. The intra- and inter-assay coefficients of variation were 7 and 9%, respectively. The luteal phase was defined as the interval between the day of ovulation and the day that progesterone concentrations declined below 1 ng/ml. The duration of the period in which progesterone levels were  $\geq 1$  ng/ml was also recorded.

The interoestrous and interovulatory intervals, the duration of the luteal phase and the period with progesterone  $\geq 1$  ng/ml were compared by ANOVA. Daily progesterone concentrations were analysed by GLM procedure of the Statistical Analysis System using an ANOVA for repeated measure (SAS, 1989). Data were presented as mean  $\pm$  S.E.M., and differences were considered to be significant when  $P < 0.05$ .

### 3. Results

#### 3.1. Interoestrous and interovulatory intervals

All control goats had interoestrous and interovulatory intervals of normal duration. The treated goats showed two different responses. Three D0 goats and one D3 goat showed “short” cycles. The others three D0 goats and four D3 goats had oestrous cycles that fell within the normal range (17.5–22 days) but the mean interval was shortened compared with the control goats (Table 1). Therefore, these goats were considered as having “shortened” cycles. Nevertheless, in the goats with shortened cycles, the length of the period with progesterone levels over 1.0 ng/ml was similar to control goats (Table 1). One D3 goat

Table 1

Interoestrous (IEI) and interovulatory (IOI) intervals and length of the period with progesterone (P4) levels over 1.0 ng/ml for controls ( $n = 7$ ) and progesterone-treated goats (CIDR) from days 0 to 5 (D0,  $n = 6$ ) or from days 3 to 8 (D3,  $n = 5$ ) postovulation<sup>a,b</sup>

Group	Length of cycle <sup>c</sup> ( $n$ )	IEI (days)	IOI (days)	P4 over 1.0 ng/ml (days)
Control	Short (0)	–	–	–
	Normal (7)	20.4 ± 0.5 a	20.0 ± 0.2 a	16.0 ± 0.5 a
D0	Short (3)	8.0 ± 0.0 b	8.0 ± 0.0 b	5.7 ± 0.3 b
	Normal (3)	18.0 ± 0.0 c	18.3 ± 0.3 c	15.7 ± 0.3 a
D3	Short (1)	10.5	11	8
	Normal (4)	18.8 ± 0.5 c	18.5 ± 0.3 c	15.8 ± 0.7 a

<sup>a</sup> Mean ± S.E.M.

<sup>b</sup> Different letters in the same column differ ( $P < 0.05$ ).

<sup>c</sup> Short: <12 days; normal: range 17.5–22 days.

had a longer cycle (26 days) and this animal was excluded from the subsequent general analyses.

### 3.2. Corpus luteum and progesterone

In control goats, one ( $n = 1$ ), two ( $n = 5$ ) or three ( $n = 1$ ) CL per interovulatory cycle were observed. The CL were detected ultrasonically between 2 and 6 days postovulation in all goats. In treated goats that developed short cycles, on days 4–5 the diameter of CL decreased and the echotexture of the luteal tissue faded. The mean progesterone concentrations decreased immediately after CIDR withdrawal in these goats. The other treated goats maintained functional CL, which diameters increased and did not differ from the CL of the control group until day 14. A decrease in the definition of the ultrasound image of luteal tissue that is associated with CL regression, as described by de Castro et al. (1999) was observed earlier in treated goats with shortened cycles (days 14.1 ± 0.5 and 15.3 ± 0.3 for D0 and D3, respectively) than in control goats (days 16.3 ± 0.3;  $P < 0.05$ ).

The daily mean serum progesterone concentrations are depicted in Fig. 1. In control goats mean progesterone concentrations increased over 1.0 ng/ml on days 2.0 ± 0.5, continued to increase until days 12.7 ± 0.4 reaching a mean of 11.7 ± 1.6 ng/ml, and then decreased below 1.0 ng/ml on days 18.0 ± 0.5.

In the treated groups mean progesterone concentrations were higher after CIDR insertion compared to control goats and remained high until CIDR withdrawal (Fig. 1). Thereafter the goats showed two different responses. In four does (D0,  $n = 3$ ; D3,  $n = 1$ ) progesterone concentrations fell rapidly below 1.0 ng/ml after CIDR removal (days 6.3 ± 0.3 and 9, respectively) and this was followed by oestrus and ovulation (short cycle). In the other seven treated does (D0,  $n = 3$ ; D3,  $n = 4$ ) progesterone remained elevated until days 15–17 and the mean concentrations did not differ from those of the controls. In these three D0 goats progesterone concentrations declined below 1.0 ng/ml earlier than in controls (days 15.7 ± 0.3 and 18.0 ± 0.5, respectively,  $P < 0.05$ ). The mean value for the four D3 goats was intermediate (days 15.8 ± 0.7). However, the length of the period with progesterone

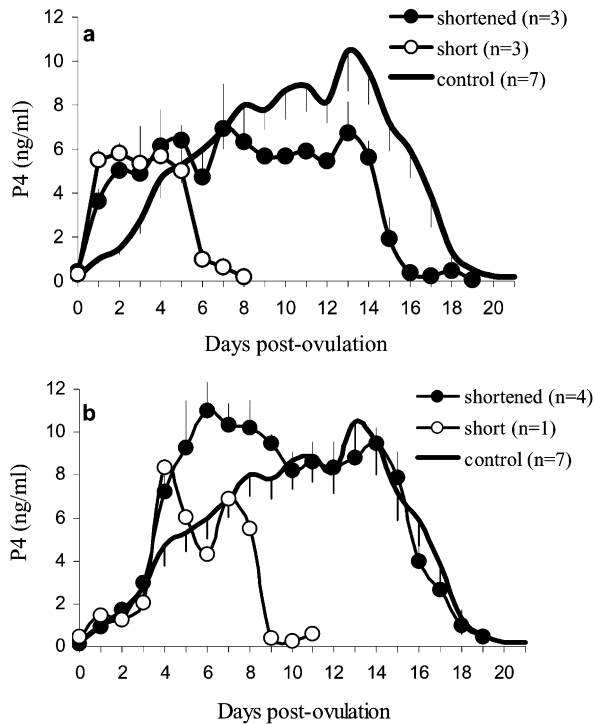


Fig. 1. Daily mean serum progesterone concentrations for goats treated for 5 days with a CIDR inserted either day 0 (a,  $n = 6$ ) or day 3 (b,  $n = 5$ ) and which developed short or shortened oestrous cycles. The progesterone profiles of control goats (Ctrl.  $n = 7$ ) were included for comparison. A significant group effect ( $P \leq 0.05$ ) was found on days 1, 2, 15 and 16 for day 0 goats with shortened cycles; days 5, 6 and 7 for day 3 goats with shortened cycles and days 1 and 2 for day 0 goats with short cycles compared with control goats.

concentrations over 1.0 ng/ml did not differ among the treated goats with shortened cycles and the control goats (Table 1).

### 3.3. Follicular dynamics

The control goats had either three ( $n = 4$ ) or four ( $n = 3$ ) follicular waves per interovulatory cycle. The interoestrous and interovulatory intervals did not differ between three- and four-wave goats. Two D0 goats with shortened cycles had four follicular waves and the other goat had three follicular waves. The four D3 goats with shortened cycles had three waves. In all D0 goats with short cycles, wave 1 was the ovulatory wave but the D3 goat with a short cycle ovulated from wave 2. The ovulatory follicles were in the growing phase when CL began to decrease in size in all goats regardless of treatment.

The D3 treated goat with prolonged interoestrous and interovulatory intervals (26 days) had a luteal phase of 23 days. The CL was recorded by ultrasonography between days 4 and 23. This goat showed four follicular waves and the ovulatory follicle emerged on day 19 and ovulated on day 26.

#### 4. Discussion

The length of the interovulatory and interestrus intervals observed in the control goats of this study are in agreement with those reported in previous studies (Chemineau et al., 1991). The duration of the luteal phase was also similar to other reports based on assays of circulating progesterone levels (Llewelyn et al., 1993).

The present results show that the elevation of progesterone concentration early during the ovulatory cycle affected the interovulatory and interestrus intervals in the goat. The insertion of a CIDR either between days 0 and 5 or between days 3 and 8 produced two responses: short cycles (less than 12 days) or shortened cycles (i.e. individual values between normal range but the mean was shorter compared with control cycles). This effect was more evident when the treatment began on day 0 as short cycle responses were more frequently found in this group (50%).

Previous studies have demonstrated that the premature elevation of progesterone during the early phase of the cycle reduces the duration of the luteal phase in other ruminants (ewes: Woody et al., 1967; cows: Ginther, 1970; Battista et al., 1984) and the present study extends these findings to the goat. The two critical responses observed (i.e. short and shortened cycles) were also described previously by Burke et al. (1994) after the insertion of a CIDR early in the cycle of cattle. Two different mechanisms may underlie both of these responses. It has been proposed that one possible reason for the premature regression of the CL is an inadequate luteotropic support after ovulation, when LH pulses are essential for CL development (Kaltenbach et al., 1968; Karsch et al., 1971) but, alternatively, a premature activation of the luteolytic mechanism could occur (Hunter, 1991; Baird, 1992).

The down regulation of endometrial progesterone receptors by progesterone is an important factor in the onset of luteolysis (Morgan et al., 1993) and progesterone treatment early in the cycle in ovarian-autotransplanted ewes (Al-Matubsi et al., 1998) and cows (Mann et al., 1998) resulted in subsequent premature luteolysis release. In the present study, goats treated on day 0 which developed shortened oestrous cycles had a shorter luteal phase than the control goats but the length of period with progesterone levels over 1.0 ng/ml (15–16 days) was similar to that observed in the controls. Thus, a normal period of progesterone priming preceded the natural PGF<sub>2a</sub> induced luteolysis. The present results suggest that in goats, as well as in the ewe and the cow, a constant period of progesterone exposure is a prerequisite for the activation of the luteolytic mechanisms by the endometrium.

In the goats with a short cycle response the serum progesterone concentrations did not allow us to determine the exact time at which the CL started to regress as both, endogenous and exogenous hormone were determined by the RIA assay used. However, according to the ultrasonic data the CL did not grow and/or regressed early in goats that showed short cycles. It may be possible that premature exposure to high progesterone levels early in the cycle caused a disruption of luteotropic factors that support the CL, inducing its premature regression and a subsequent short cycle. LH is the major luteotropic agent that promotes CL functionality provided that the process of luteolysis has not already been initiated (Armstrong et al., 1983; Kaltenbach et al., 1968). Hypophysectomy in sheep (Karsch et al., 1971) caused regression of the CL, an effect that was reversed by exogenous LH; and immunosuppression of LH also inhibited CL functioning (Hoffman et al., 1974). It is known that high progesterone levels decrease LH concentrations and parti-

cularly LH pulsatility in sheep and cattle. It was proposed that progesterone-treated ewes during the early cycle reduced the support by LH rendering the CL more sensitive to PGF<sub>2a</sub> (Ottobre et al., 1980). Although, similar studies have not been conducted in the goat, one possible explanation of the occurrence of short cycles is the effect on LH secretion induced by the progesterone administered. As LH concentrations were not determined in the present study we can only speculate about this phenomenon. Alternatively, a premature release of PGF<sub>2a</sub> in response to progesterone treatment may also have occurred in the goats with a short cycle response. This effect has been reported in cows after progesterone treatment during the early metoestrus (Garret et al., 1998).

In conclusion, premature elevation of concentrations of progesterone during the oestrus cycle of the goat promoted either short or shortened oestrus cycles. The underlying mechanisms for this effect remain unclear and warrant future investigation.

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