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Sudden introduction of bucks during the late luteal phase of isolated female goats induces a biphasic change in progesterone concentrations

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Abstract

The aim was to determine the progesterone profile after the introduction of bucks during the advanced luteal phase of does. Fourteen does received vaginal sponges impregnated with 40 mg fluorogestone acetate for 12 days, and a luteolytic dose of a prostaglandin analogue (75 µg of D-cloprostenol) 2 days before sponge removal. Fifteen days after sponge withdrawal one buck was introduced in one of the pens (BE group; n = 6), while the female goats in the other pen remained as controls (CON group; n = 8). The buck was replaced every 24 h, alternating their presence until the end of the experiment. Serum progesterone levels were used to monitor ovarian activity. Progesterone concentration from day 14 to 20 varied with time ($P < 0.0001$), and there was an interaction between treatment and day ($P = 0.02$). While progesterone concentration increased from day 15 to day 16 in BE does ($P = 0.01$), there were no changes in CON does on those days ($P = 0.2$). On the other hand, progesterone concentrations decreased in BE does from day 18 to day 19 ($P = 0.02$), without changes in CON does ($P = 0.6$). Finally, there was a sharp decrease from day 19 to day 20 in both BE ($P = 0.0009$) and CON ($P < 0.0001$) does. Overall, our results demonstrated that the introduction of bucks during the late luteal phase of isolated does can induce changes in the progesterone pattern, showing an early increase followed by a pronounced withdrawn.

Keywords: estrous cycle, luteolysis, male effect, pheromones, socio-sexual signals.

Introduction

In sheep and goats the introduction of males to a flock of previously isolated anestrous females (the ram effect and buck effect, respectively) induces ovulation, estrus, and might end in out-of-season pregnancies (for reviews, see: Ungerfeld *et al*., 2004; Delgadillo *et al*., 2009). As happens in ewes (Martin and Scaramuzzi, 1983), the introduction of males induces a rapid increase in LH pulsatility in both anestrous (Chemineau *et al*., 1986) and cyclic (Hawken *et al*., 2009) goats. A similar increase is observed in ewes treated with progestagens (Evans *et al*., 2004) or pregnant ewes (Al-Gubory, 1998).

As LH pulsatility induces an increase in estradiol concentrations, and an estradiol increase is a first step to trigger luteolysis (Hixon and Flint, 1987), it may be expected that the introduction of the males induce luteolysis. Supporting this hypothesis, Chemineau (1983) observed a bimodal estrus response after introducing bucks to cyclic does, suggesting that luteolysis was provoked in part of the flock. Also in goats, Mellado and Hernández (1996) observed an important concentration of estrus in cyclic goats stimulated by males, which may be a consequence of the advancement of the luteolysis in some does. Due to this possible luteolytic action of the ram effect, Ungerfeld (2011) and Meilán and Ungerfeld (2014) aimed to partially substitute the administration of PGF2α in estrous synchronization treatments. Although in both studies there was a positive response of the introduction of rams, this was observed only in a small percentage of the ewes.

On the other hand, Valencia *et al*. (2010) introduced bucks in different periods of the estrous cycle without changes in cycle length. However, it should be considered that as males induce a rapid increase of LH pulsatility, and LH stimulates secretion of progesterone by the corpus luteum (see review: Stouffer, 2006), there may be an early response enhancing corpus luteum activity. It should also be considered that in a normal estrous cycle the increase of PGF2α is observed 12 to 48 h after the increase of estradiol (Ford *et al*., 1975). Therefore, it may be expected not to observe a luteolytic response immediately after the introduction of the males, but it may be expected to observe it later. Thus, our aim was to determine the progesterone profile after the introduction of bucks during the advanced luteal phase of does.

Material and Methods

Animals and management

The experiment was conducted over a two-month period beginning in late August, a period coincident with the normal breeding season for goats at this latitude (19º N). Animals were housed in an open-sided barn under natural lighting and were fed with a maintenance diet of forage and commercial feed with 14% crude protein. Water and minerals were offered *ad libitum*.

Animals were two one-year old sexually active Saanen bucks, weighing 45.0 ± 1.4 kg (mean ± SEM), and

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14 cyclic, non-lactating 2.5 year-old does (34.0 ± 0.9 kg) from the same breed, maintained at the facilities of the Universidad Autónoma del Estado de Morelos, Mexico. Before the initiation of the experiment, all females remained completely isolated from males for at least six months. Does were housed in two different pens 10 x 5 m each separated 100 m from each other, with 6 and 8 does/pen, and were maintained as single groups until the end of the study.

Vaginal sponges containing 40 mg fluorogestone acetate (Chronogest, Intervet, Mexico) were placed in all does for 12 days, and an intramuscular injection of a PGF2α analogue (75 µg of D-cloprostenol, Prosolvin – C, Intervet, Mexico) was applied two days before sponge removal.

On day 15 after sponge withdrawal one buck was introduced in one of the pens (BE group; n = 6), while does in the other pen remained as controls (CON group; n = 8). Each buck was replaced every 24 h, alternating their presence until the end of the experiment. The bucks were kept in a pen contiguous to the BE group.

Response recording

Ovarian activity was monitored through serum progesterone levels. Blood samples were collected daily from each doe in both groups, from sponge withdrawal to 22 days. Blood was collected by jugular venipuncture and evacuated in glass tubes from 9:00 to 9:30 h. Samples were immediately cooled and held in ice water until serum separation by centrifugation within 60 min of collection. The samples were frozen at -2ºC until analysis. Progesterone concentrations were determined using commercial coated tube RIA kits (Coat-A-Count Progesterone; Siemens Medical Solutions Diagnostics. Los Angeles, CA). The sensitivity of the assay was 0.1 ng/ml and the mean intra- and inter-assay coefficients of variation calculated from the low and high control samples (1.71 ng/ml and 39.2 ng/ml respectively) were 6 and 8%, respectively. The existence of ovulation was assumed when progesterone concentrations were above 1 ng/ml (Ravindra and Rawlings, 1997).

Statistical analysis

The length of the luteal phase in BE and CON goats was compared by an ANOVA. The progesterone concentration pattern from 14 to 22 days after sponge withdrawn of BE and CON goats was compared with the mixed procedure of SAS including the treatment, the day, and the interaction of treatment and day as fixed effects, and the goat into each group as fixed effects.

Results

Length of the luteal phase was similar in BE and CON does (15.5 ± 0.2 *vs*. 15.1 ± 0.3 days, respectively). Progesterone concentrations were below 1 ng/ml in all does 20 days after sponge withdrawal.

Progesterone concentration from day 14 to 20 varied with time (P < 0.0001), and there was an interaction between treatment and day (P = 0.02; Fig. 1). While progesterone concentration increased from day 15 to day 16 in BE does (P = 0.01), there were no changes in CON does on those days (P = 0.2). On the other hand, progesterone concentrations decreased in BE does from day 18 to day 19 (P = 0.02), without changes in CON does (P = 0.6). Finally, there was a sharp decrease from day 19 to day 20 in both BE (P = 0.0009) and CON (P < 0.0001) does.

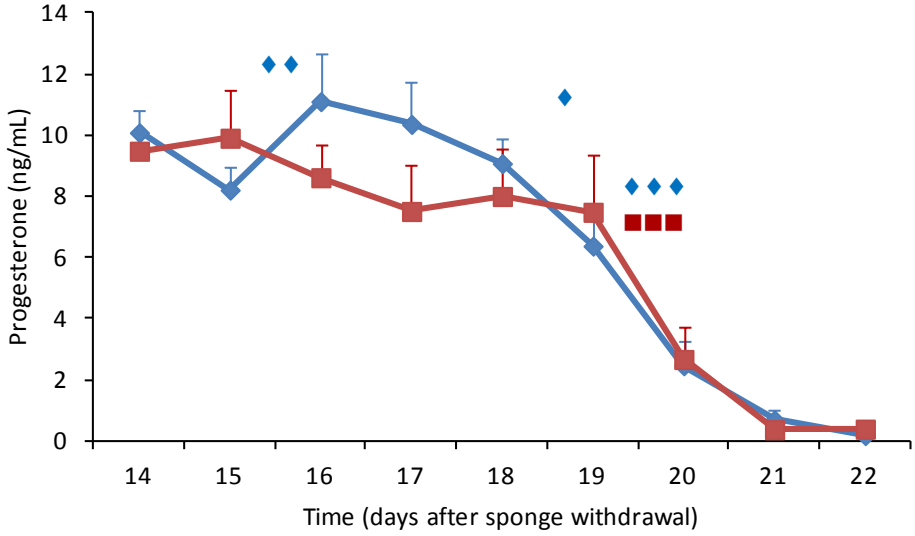

Figure 1. Progesterone profiles in does that remained isolated (–■–) or were stimulated by bucks (–◆–) 13 days after the end of an estrous synchronization treatment. The symbols indicate when the differences between the previous and the following day were significant. While ■ indicates differences in isolated does, ◆ indicate differences in stimulated does. One symbol corresponds to P < 0.05; two symbols to P < 0.01, and 3 symbols to P < 0.001.

Discussion

Our results demonstrate that the sudden introduction of bucks modified the pattern of progesterone secretion in cyclic does. The changes induced by males are strong enough to be detected even with the low number of animals used. The response of stimulated does was biphasic, with an initial increase, probably due to the stimulation of the corpus luteum by the increase in LH pulses (for review see: Stouffer, 2006). This agrees with the increase observed in progesterone concentration by Valencia *et al*. (2010) after introducing bucks to isolated does 7 or 12 days after the end of an estrous synchronization treatment. After this increase we observed an earlier progesterone withdrawal, which did not end in an earlier luteolysis because it began with greater progesterone concentrations. This second response advancing the luteolytic process was probably due to the estradiol secretion induced by the males. Overall, although this model did not modify the estrous cycle length, the introduction of bucks may be potentially used to trigger an earlier luteolysis. In effect, it would be interesting to determine if similar effects may be obtained in an earlier moment of the estrous cycle.

The observed bimodal progesterone pattern is useful to explain results of previous studies in which they aimed to use the ram effect during the late luteal phase to substitute a whole (Ungerfeld, 2011) or half PGF2α dose (Meilán and Ungerfeld, 2014). In those studies, only estrous was used to determine the response. According to our present results, although the male effect may have triggered a luteolytic response, this was probably not effective in shortening the estrous cycle length as bucks were introduced early before the onset of the spontaneous luteolysis. It should also be considered that in general terms, goats are more sensible than ewes to socio-sexual stimuli, so it may be possible to observe a greater response in does than in ewes. However, Valencia *et al.* (2010) did not observe differences on estrous cycle length after introducing bucks on days 7, 12, or 17 days after the end of a synchronization estrous treatment. In this sense, it may be possible that the estradiol increase induced by the introduction of the bucks during the early or mid-luteal phase is not enough to induce luteolysis alone. It should be considered that most goat breeds are poliovulatory, making it more difficult to trigger luteolysis with only the introduction of the bucks when the corpora lutea are fully active. In this sense, to obtain positive responses during the early or mid-luteal phases, it may be interesting to administer a mild PGF2α dose 24-36 h after the introduction of the males, as was proposed by Meilán and Ungerfeld (2014). This would synchronize the spontaneous secretion and the pharmacological application of PGF2α.

Overall, our results demonstrated that the introduction of bucks during the late luteal phase of isolated does can induce changes in the progesterone pattern, showing an early increase followed by a pronounced withdrawn.

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