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Progesterone (P4), luteinizing hormone (LH) levels and ovarian activity in postpartum Santa Inês ewes subject to a male effect

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Abstract. This study aimed to establish P4 and luteinizing hormone (LH) levels and ovarian activity as approaches to monitor the cyclicity of ewes under postpartum anestrus after the male effect approach. Santa Inês females ($n = 66$) were evenly distributed into experimental groups where they were brought into contact with an intact male during the postpartum period of 35 to 40 days (T1) and 55 and 60 days postpartum (T2). Ewes were isolated from males for 30 days before the onset of the experiment. Estrus events were detected in 93.30% (T1) and 100 % (T2) of females. Mean P4 concentrations (η g mL^{-1}) before and after mating were 0.53 ± 0.17 and 4.55 ± 0.24 (T1) and 0.73 ± 0.06 and 4.90 ± 0.11 (T2), respectively, and concentrations were found to be lower ($P < 0.05$) before contact between genders. Preovulatory peaks of LH (η g mL^{-1}) were evaluated at 42 (T2) and at 80 h (T1) after exposure to males, with mean ovulatory follicles of 7.90 ± 0.31 (T1) and 8.50 ± 0.30 mm (T2) and a mean number of ovulations of 1.50 ± 0.54 (T1) and 1.60 ± 0.51 (T2). Pregnancy rates were 85.70 % (T1) and 93.3 % (T2), with no difference ($P > 0.05$) between groups. Results showed that the male effect was effective for inducing and concentrating the occurrence of estrus in postpartum ewes that had lambed within 35 to 60 days previously. Moreover, preovulatory LH peaks occurred within 80 h after physical contact between genders, which led to follicle luteinization and increased P4 concentration, without compromising pregnancy rates. The male effect can be used with postpartum ewes that had lambed within 35 to 40 days previously in order to decrease time between deliveries.

1 Introduction

Traditional sheep production in South America is predominantly sustained by production systems in extensive areas with low reproductive efficiency (Rubianes and Ungerfeld, 2002). Moreover, seasonal reproduction systems and long postpartum anestrus periods are factors that affect sheep production by reducing overall lambing rates. Shorter periods of sexual inactivity, the anticipation of the onset of the breeding season and increased prolificacy are economically relevant factors that allow profitability in such commercial settings (Oliveira et al., 2015; Tenório Filho et al., 2016; Ferreira-Silva et al., 2016). These factors are particularly true when carried out under adequate management practices and when those technologies are of low cost and simply executed (Simplício, 2008).

Hair sheep breeds have the potential to produce high-quality meat and skin; they are also hardy animals and of great adaptive capacity to tropical regions, such as the northeast of Brazil (Machado et al., 1999). From a productive standpoint, the Santa Inês breed, which originated from crosses of exotic and Brazilian native breeds, is characterized by a large size, where ewes weigh from 40 to 60 kg and rams from 80 to 100 kg, although they may reach 120 kg (Figueiredo et al., 1983). From a reproductive standpoint, ewes are continuously polyestrous, with prolificacy varying from 1.3 to 1.4, and have maternal ability (Ferreira-Silva et al., 2016, 2017).

Sheep production can be improved by genetic selection for ewes of improved reproductive efficiency, with lower age at first lambing, shorter time between deliveries and increased prolificacy (Notter and Copenhaver, 1980; Azzarini, 2004). The male effect alone (Sasa et al., 2011; Caldas et al. 2015a, b) or combined with pharmaceuticals (Knights et al., 2001; Ungerfeld et al., 2004; Monreal et al., 2009; Ferreira-Silva et al., 2017) is a simple, effective and low-cost approach to reducing the duration of postpartum anestrus without affecting pregnancy rates (Caldas et al., 2015a, b).

The sudden introduction of males into a female flock of sheep where the animals are in anestrus results in an immediate increase in luteinizing hormone (LH) pulse frequency and amplitude at intervals of 54–72 h (Knight et al., 1983) followed by ovulation (Martin et al., 1983; Moraes, 1991; Thimonier, 2000). This variation in LH pulses may be due to silent estrus behavior in ewes under anestrus (Knight et al., 1983; Thimonier, 2000). Thus, depending upon the resulting type of corpus luteum that is formed, females may have displayed short or normal estrous cycles (Caldas et al., 2015a, b), accompanied by an increase in P4 concentration (Sasa et al., 2011).

In face of the ongoing demand to reduce the interval between deliveries to increase sheep reproductive efficiency, the male effect could be used to shorten anestrus postpartum in ewes. Furthermore, to our knowledge, there is no report that used the male effect for this purpose in hair sheep, and the hormone levels and ovarian activity within this period remain to be described. This study aimed to evaluate changes in the cyclicity of anestrus postpartum Santa Inês ewes after the introduction of an intact male by determining P4 and LH profiles and ovarian activity.

2 Material and methods

Experiments were conducted in Escada, Pernambuco state, Brazil. The geographic coordinates are 08°21′33″ S, 35°13′25″ W; the altitude is 109 m, the mean annual temperature 24.4 °C and the mean annual rainfall 1763 mm^3. Climactic conditions are tropical and semi-humid, with a rainy period from May to August.

Animals were raised on pastures during daylight hours and kept in a barn during the late afternoon. Nutrition was based upon cultivated (*Brachiaria humidicola*, *Pennisetum purpureum*) and native pastures (*Paspalum maritimum*, *Chloris orthonton*, *Cynodon dactylon*, *Brachiaria tunnergrass*). Mineral salt and water were offered ad libitum.

Postpartum ewes of 2 to 3 years of age were initially evaluated for body condition score and were preselected if they displayed a score of 2 or 3, as described by Caldas et al. (2015a, b). The anestrus condition was evaluated by reproductive tract ultrasonography following Santos et al. (2004). Selected ewes were identified with numbered plastic ear tags and colored neckband in order to ease management. Male effect preconditioning was established by isolating ewes from rams for 30 days at a distance of 10 m, which avoids physical contact between genders as previously described by Caldas et al. (2015b). Santa Inês rams ($n = 2$) were preselected according to fertility records and were subject to an andrology exam a week before the onset of the experiment.

Cyclicity status was determined based upon P4 concentrations in addition to ultrasonography. Blood samples were collected on days 10, 20 and 30 during the male effect preconditioning period. Blood plasma was stored before analysis at −20 °C. Progesterone (P4) concentration was determined in duplicates using radioimmunoassay. Females were considered in anestrus when they displayed serum concentrations lower than 1 η g mL^{-1} in two consecutive samples as described by Morales et al. (2003). After P4 concentration analysis, ewes ($n = 66$) were allocated to two groups: T1 (35–40 days after lambing) and T2 (55–60 days after lambing).

Rams were marked with a mixture of grease and ink (4 : 1) around the sternum and then introduced to the ewe flocks, with a male-to-female ratio of 1 : 33. Rams were marked with different ink colors and exchanged between ewe flocks on days 10, 20 and 30 of the breeding season (BS).

Blood plasma LH analysis was determined by duplicate double-antibody radioimmunoassay, following De St Jorre et al. (2012), and the limit of detection was 0.27 η g mL^{-1}; the intra-assay and inter-assay coefficients of variation were 3.5 and 8.74 %, respectively. Variations in LH levels were considered significant when they increased by 20 η g mL^{-1} or more for two consecutive measurements, as suggested by Martin et al. (1983). Three ewes from each group were randomly chosen for LH analysis after the introduction of rams. Blood sampling for LH analysis was at 4 h intervals and performed during a period of 4 days after the introduction of rams, as described by Chanvallon et al. (2011) and Fabre-Nys et al. (2015, 2016). Moreover, ewes used for LH evaluation were excluded from further analysis in order to avoid any effect of excessive handling on estrus and pregnancy rates.

In order to estimate ovulation rates on ewes after mating, P4 concentration was also measured on days 10, 20 and 30 after the onset of the male effect as described above for LH analysis. Ewes were considered to have entered a cycle when they displayed a P4 concentration of 1 η g mL^{-1} or higher in two consecutive samples.

Ovarian activity was evaluated daily by ultrasonographic exams after estrous detection in six females of each group. The same technician performed all exams and the moment

Table 1. P4 concentrations in Santa Inês postpartum ewes in anestrus that had delivered within 35 to 40 (T1) and 55 to 60 days (T2) previously and were further subject to the male effect.

Group	Animals	P4 concentration (η g mL^{-1})	
	(n)	Before male effect ($\overline{x} \pm s$)	After male effect ($\overline{x} \pm s$)
T1	30	$0.53 \pm 0.17^{a,c}$	$4.55 \pm 0.24^{b,c}$
T2	30	$0.73 \pm 0.06^{a,d}$	$4.90 \pm 0.11^{b,d}$

Different superscripts ^{a,b} letters in the same row and superscripts letters ^{c,d} in the same column denote statistical difference ($P < 0.05$).

Table 2. Mean values for follicular diameter (mm) and ovulation in Santa Inês postpartum ewes in anestrus that had delivered within 35 to 40 (T1) and 55 to 60 (T2) days previously and were further subject to the male effect.

Group	Ovarian activity			
	Large follicle diameter ($\overline{x} \pm s$)	Second large follicle diameter ($\overline{x} \pm s$)	Follicular diameter ($\overline{x} \pm s$)	Ovulations ($\overline{x} \pm s$)
T1	7.9 ± 0.31^a	7.6 ± 0.41^a	7.8 ± 0.38^a	1.5 ± 0.54^a
T2	8.5 ± 0.30^b	8.0 ± 0.25^a	8.3 ± 0.37^b	1.6 ± 0.51^a

Different superscript letters on same column denote statistical difference ($P < 0.05$).

Table 3. Pregnancy rates of Santa Inês postpartum ewes in anestrus that had delivered within 35 to 40 (T1) and 55 to 60 (T2) days previously and were further subject to the male effect.

Group	Pregnancy per service		
	First n/n (%)	Second n/n (%)	Total n/n (%)
T1	18/20 (90.0)	6/8 (75.0)	24/28 (85.7)
T2	24/25 (96.0)	4/5 (80.0)	28/30 (93.3)

of ovulation was defined according to Tenório Filho et al. (2007).

Ewes were observed twice daily for estrus (06:00 and 16:00 LT) by trained technicians during a breeding season (BS) of 35 days. Estrus events were considered synchronized when detected within the initial 5 days of the BS. Pregnancy diagnosis was made by ultrasonography on days 35 and 60 after mating as described by Santos et al. (2004).

Parametric variables were submitted to analysis of variance (ANOVA) and compared by the Student–Newman–Keuls (SNK) test with the System for Statistical Analysis (SAEG) software, with results presented as means and standard deviation ($\overline{x} \pm s$). Nonparametric variables were evaluated using the chi-square test and presented as percentages (%). Differences were considered significant when displayed probabilities were lower than 5 %.

3 Results

Concentrations of P4, which were assessed before BS onset in order to determine the cyclicity status of postpartum ewes are summarized in Table 1. Mean P4 concentrations were lower than 1 η g mL^{-1}, indicating that all ewes were in anestrus before BS, irrespectively of the postpartum period. However, after the male effect, ewes cycled and ovulated, as demonstrated by increased P4 levels ($P < 0.05$).

Concentrations of LH were also measured after ewes were subject to the male effect. Despite varying postpartum periods, the male effect induced LH preovulatory peaks between 52 and 80 h after the onset of the BS (Fig. 1).

The incidence of estrus during the BS was also determined (Fig. 2). Estrus events were detected until day 33 of the BS. Estrus events were detected in 93.30 and 100 % of ewes in the T1 and T2 groups, respectively. An increased incidence of estrus was observed in T2 within the initial 10 days of the BS. First estrus occurred between days 1 and 33, with the mean for first estrus being 15.45 ± 10.36 (T1) and 9.25 ± 6.41 (T2) days post-lambing. The overall incidence of synchronized estrus was 23 % for all ewes, with 26 % in T1 and 20 % in T2, with no difference between groups ($P > 0.05$).

Ovarian activity was monitored in order to correlate it with estrus and pregnancy rates (Table 2). Large follicles ($P < 0.05$) were detected on T2, but the second large follicle did not differ between groups. The mean number of ovulations was similar between groups ($P > 0.05$). Pregnancy rates were determined after first and second mating throughout the BS (Table 3), but no difference was observed ($P > 0.05$) between groups.

4 Discussion

Monitoring reproductive activity by P4 concentration has been used to detect anestrus in ewes, in relation to seasonal factors or postpartum condition (Sasa et al., 2011). As described here, mean P4 concentrations before the male effect demonstrated that ewes were in anestrus, based upon P4 levels previously described by Morales et al. (2003). However, the exposure of ewes to the male effect, irrespective of the postpartum period, led to a significant increase in P4 levels, which is in accordance with previous reports (Sasa et al., 2002).

There is strong evidence correlating P4 concentrations to sexual behavior in anestrus ewes, as P4 stimulates receptivity to rams and increases estrous behavior (Fabre-Nys and Martin, 1991a, b; Caraty and Skinner, 1999). The total incidence of estrus was similar between groups. However, most estrus events within T2 were concentrated in the initial 10 days of the BS, in agreement with their higher P4 concentrations. Since all ewes were in anestrus prior to treatment, it was ex-

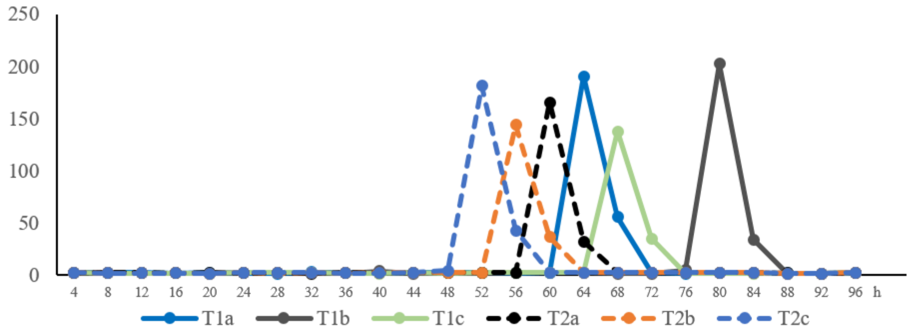

Figure 1. Individual LH concentration (η g mL^{-1}) during the period of 4 to 96 h after the onset of the male effect in Santa Inês postpartum ewes in anestrus ($n = 6$) that had delivered within 35 to 40 days (T1) and 55 to 60 days (T2) previously.

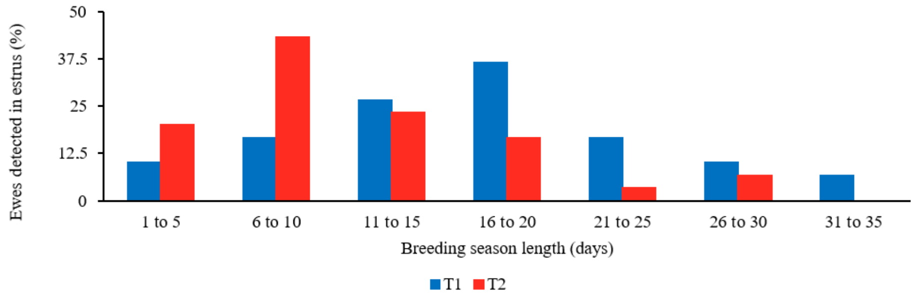

Figure 2. Distribution of estrus events in Santa Inês postpartum ewes in anestrus after being subject to the male effect and 35 to 40 days (T1) and 55 to 60 days (T2) after delivery.

pected that, irrespective to the number of days postpartum, ewes would display a similar responsiveness to the male effect. However, more encouraging results were described with cycling ewes (Caldas et al., 2015a, b) for estrus incidence within the initial 10 days of the BS and estrus synchronization within the initial 5 days of the BS.

The presence of P4 is required to induce GnRH production (Caraty and Skinner, 1999), which is responsible for the subsequent LH preovulatory peak (Martin et al., 1983). The data described here show that the LH preovulatory peak was induced approximately 30 h in advance in ewes that showed higher P4 concentrations before the male effect. Moreover, these ewes were under prolonged postpartum anestrus, a fact that may have contributed to this observation, since these ewes were expected to show higher basal LH levels than ewes under more recent postpartum periods. This hypothesis is in accordance with Martin et al. (1980), who found that higher basal LH levels before interaction between genders increased responsiveness to the male effect. Despite differences in LH preovulatory kinetics, mean LH concentrations were similar between groups and all ewes showed LH preovulatory peaks within 80 h after the male effect onset, as previously described by Oldham et al. (1979). This similarity between results is in agreement with similar incidences of short estrus cycles and the number of ovulations between groups. Due to these facts, the determination of LH levels at 4 h intervals was efficient to capture the preovulatory peak as described in other reports (Chanvallon et al., 2011; Fabre-Nys et al., 2015).

Although LH preovulatory peaks occurred later in ewes with more recent delivery, it may not have contributed to oocyte competence during follicle growth or luteinization, even after the use of exogenous P4 in association with the male effect (Skinner et al., 2000). Accordingly, the large follicle diameter was large in ewes under prolonged postpartum period conditions and did not affect pregnancy viability, since P4 produced after follicle luteinization was sufficient to maintain full-term pregnancies at similar rates.

5 Conclusions

The male effect is efficient to induce and concentrate estrus within a small window of time in postpartum ewes within 35 to 60 days after lambing. Under these conditions, the LH preovulatory peak occurs within 80 h, which ultimately leads to follicle luteinization and further increases in P4 synthesis. Moreover, postpartum ewes can be subjected to the male effect in order to reduce the time between deliveries, with no effect on overall pregnancy rates.

Data availability. The research data described in the article are available for those who request it. Any concerns about our work will be addressed accordingly in due time.

Competing interests. The authors declare that there is no conflict of interest and are available to provide any clarification.

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This research was performed after evaluation and approval of the Ethics Committee of the Faculdade Pio Décimo, Aracaju-Se, Brazil, with protocol no. 08/12.

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