

MALE EFFECT ASSOCIATED WITH SUCKLING INTERRUPTION ON THE REPRODUCTIVE PERFORMANCE OF SANTA INÊS EWES¹

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ABSTRACT: This study aimed to evaluate the effect of temporary suckling interruption associated with the male effect on reproductive performance of pluriparous Santa Inês ewes. The females were kept apart from the males for 60 days and then randomly distributed into three treatments associated with the male effect (DT0, DT24 and DT48); in DT0, there was no suckling interruption; in DT24, suckling was interrupted for 24 hours, and in DT48, suckling was interrupted for 48 hours. Estrous distribution was observed within 31 (DT0), 27 (DT24) and 38 (DT48) days of the breeding season. Estrous synchronization up to the 5th day of the mating season was observed in 15% (DT0), 30% (DT24) and 25% (DT48) of the females, with no difference among treatments. Estrous percentages were 90% (DT0), 100% (DT24) and 100% (DT48), with no difference among treatments. Pregnancy rates were 38.4% (DT0), 60.0% (DT24) and 45.0% (DT48) with no difference among treatments. Prolificacy was 1.43 (DT0), 1.17 (DT24) and 1.22 (DT48) and did not differ between treatments. In conclusion, temporary suckling interruption associated with the male effect is efficient to induce estrous but not to synchronize estrous or increase the pregnancy rates and prolificacy of Santa Inês ewes during a 45-day breeding season.

Keywords: anestrous, biostimulation, reproduction, sheep.

EFEITO MACHO ASSOCIADO AO DESMAME TEMPORÁRIO NO DESEMPENHO REPRODUTIVO DE OVELHAS SANTA INÊS

RESUMO: O estudo teve como objetivo avaliar o efeito do desmame temporário associado ao efeito macho sobre o desempenho reprodutivo de ovinos Santa Inês. As fêmeas foram mantidas distantes dos machos por 60 dias e aleatoriamente distribuídas em três tratamentos associados ao efeito macho (DT0, DT24 e DT48), no qual em DT0, não houve interrupção da amamentação; em DT24, amamentação interrompida por 24 horas e em DT48, interrupção da amamentação por 48 horas. Distribuição de estro foi observada em 31 (DT0), 27 (DT24) e 38 (DT48) dias da estação de monta. Sincronização de estro até o quinto dia da estação de monta foi observada em 15% (DT0), 30% (DT24) e 25% (DT48) das fêmeas, não havendo diferença entre os tratamentos. Percentagens de estro foram de 90% (DT0), 100% (DT24) e 100% (DT48), não havendo diferença entre os tratamentos. As taxas de prenhez foram de 38,4% (DT0), 60,0% (DT24) e 45,0% (DT48), sem diferença entre os tratamentos. A prolificidade foi de 1,43 (DT0), 1,17 (DT24) e 1,22 (DT48), e não diferiu entre os tratamentos. Em conclusão, o desmame temporário associado ao efeito macho é eficiente na indução do estro, embora não seja eficiente na sincronização de estros e não aumente as taxas de gestação e prolificidade das ovelhas Santa Inês durante uma estação de monta de 45 dias.

Palavras-chave: anestro, bioestimulação, ovinos, reprodução.

INTRODUCTION

Ovine production is a socially and economically relevant activity on a global scale, but inadequate management practices hamper reproductive performance (HAYES, 1971), most notably pregnancy rates and prolificacy.

The male effect is a biostimulation process mediated by pheromones that promotes endocrine reactions that lead to changes in the reproductive behavior of females in different species (KNIGHT *et al.*, 1983; REKWOT *et al.*, 2001; DELGADILLO, 2011; LOPEZ-SEBASTIÁN *et al.*, 2014). The endocrine basis of the male effect has been mainly investigated in sheep, where sudden introduction of fertile rams alters tonic gonadotrophin secretion in ewes, culminating in a preovulatory LH peak and ovulation (MARTIN *et al.*, 1986; COHEN-TANNOUJJI *et al.*, 1989). Moreover, the male effect is successfully used in estrous synchronization and ovulation induction in sheep (KEISLER and BUCKRELL, 1997). Although estrous synchronization is more efficient under chemically based protocols, fertility rates are higher using the male effect.

The inhibitory effect of suckling on reproduction has been linked to the extension of first estrous after delivery due to GnRH release inhibition and reduction of LH pulse frequency and amplitude (WILLIAMS, 1990; BROWNING JR. *et al.*, 1994). The association between temporary suckling interruption and the male effect in sheep induces a rapid increase in both the frequency and amplitude of LH pulses (POINDRON *et al.*, 1980; CHEMINEAU *et al.*, 1986), stimulating pituitary activity that sustains follicle growth, followed by a preovulatory LH peak that leads to ovulation (CHEMINEAU, 1985; MARTIN *et al.*, 1986; CHEMINEAU, 1987; SHIVELY and WILLIAMS, 1989).

Reduction in the conception interval is fundamental to increase the reproductive performance of a herd while providing nutritional needs (DUNN and MOSS, 1992; FORCADA *et al.*, 1992) and modifying suckling regimens (SHEVAH *et al.*, 1975; RHIND *et al.*, 1980).

Temporary suckling interruption in conjunction with the male effect may increase the reproductive performance of the herd and the profitability of the activity, culminating in end products (meat, milk, and derived products) free of residual contamination by exogenous hormones. Therefore, this work was conducted to evaluate the effect of temporary suckling interruption and the male effect on estrous synchronization and fertility of lambing Santa Inês ewes.

MATERIALS AND METHODS

This study was conducted in Pernambuco state, Brazil. The geographic coordinates are latitude 08° 04' 25" South, longitude 37° 15' 52" West. The altitude is 558 m, and the region has semi-arid weather conditions, a mean annual temperature of 25° C, and an annual rainfall of 635 mm³.

Pluriparous lambing females (n = 60), 24 to 36 months of age, that had delivered within 45 to 60 days, were selected for the study. The ewes were fed with native and cultivated (*Cenchrus ciliaris*, L.) pastures. The animals had free access to mineral salt and water.

The day before experiment onset, the females were weighed and identified with ear tags. Their ovaries were scanned by ultrasound to identify the *corpus luteum*, and blood samples were taken for progesterone (P4) quantification using the chemiluminescence method. The females were scored as cycling when P4 levels reached 1 ng/mL, as described by STELLFLUG *et al.* (1997).

The females were randomly distributed into experimental groups (DT0; DT24; DT48), considering the age, date of delivery, weight, and presence of a *corpus luteum*. In DT0 (n = 20) no suckling interruption was performed (control); in DT24 (n = 20), suckling was interrupted for 24 hours; and in DT48 (n = 20), suckling was interrupted for 48 hours.

Males of proven fertility (n = 3), 24 to 48 months of age, were initially kept in individual pens at a distance of 300 m from the herd of females to avoid any visual, olfactory and auditory contact for 60 days. The rams were fed cultivated pasture (*Pennisetum purpureum*, Schum.) and 0.2 Kg of concentrate supplement (Durancho®) and 0.2 kg of grain corn (*Zea mays*, L) per animal/day.

The day before the start of the mating season, the bucks were subjected to an andrology evaluation, as recommended by CBRA (1998), to confirm their reproductive status. Upon being introduced randomly to the flock, the buck was marked with a mixture of grease and check dye (4:1) every ten days in the breastbone region to score females in estrous. Estrous visual detection was performed daily at 6:00 am and at 4:00 pm for one hour by trained personnel.

Estrous was considered as synchronized by the male effect until Day 5 of the breeding season, as reviewed by FIERRO *et al.* (2013) for animals treated with prostaglandin. Pregnancy diagnosis was performed by rectal ultrasound exam on day 60 after the last mating (SANTOS *et al.*, 2004).

Statistical analysis was performed by the Kruskal-Wallis test and Fisher's Exact test, and differences of 5% were considered significant.

RESULTS

Estrous synchronization was observed in 49 (76.56%) females, 14 (70.00%) in DT0, 18 (81.81%) in DT24 and 17 (77.27%) in DT48, with no difference between the groups ($P > 0.05$). Figure 1 shows estrous dispersion until day 38 in a breeding season of 45 days. It is possible to note a higher estrous concentration until day 10, with no difference between groups ($P > 0.05$). Until day 5, 23.3% of all females had synchronized estrous, 15% in DT0, 30% in DT24 and 25% in DT48 ($P > 0.05$). In Figure 1, a high concentration of estrous between days 21 and 25 of the breeding season is also noticeable within females that had already cycled.

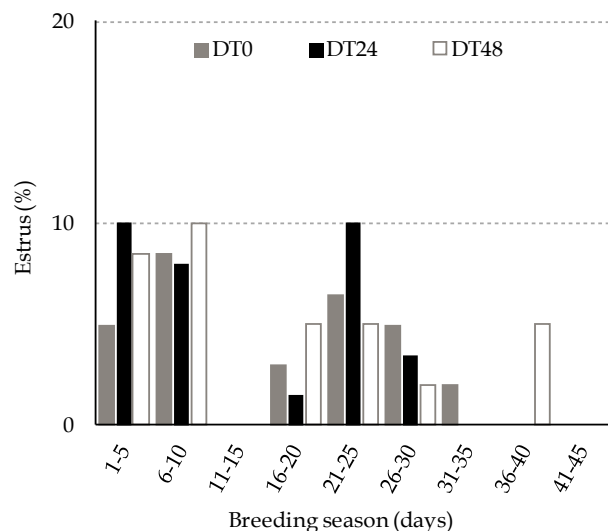


Figure 1. Estrous distribution in Santa Inês ewes under male effect and suckling interruption during a breeding season of 45 days.

During the experiment, the most ewes that cycled within the groups varied from 90.00% to 100%, where 35 (58.33%) had a single estrous and 23 (38.33%) had two estrous cycles (Table 1) ($P > 0.05$). Of all the females with two estrous cycles, 7 (35.00%) exhibited short estrous cycles.

The pregnancy rates were 35.00% in DT0, 60.00% in DT24 and 45.00% in DT48, with no difference between the groups ($P > 0.05$) (Table 2). Moreover, 9

(15.00%) females conceived on the first estrous, and DT24 was superior ($P < 0.05$) to DT48 but similar to DT0 ($P > 0.05$). On the second estrous, 19 (31.66%) conceived, and DT48 was superior to DT0 ($P < 0.05$) but similar to DT24 ($P > 0.05$).

Table 1. Estrous types in Santa Inês ewes following exposure to the male effect without suckling interruption (DT0), suckling interruption for 24 hours (DT24), or suckling interruption for 48 hours (DT48) during a breeding season of 45 days

Number of estrous	Suckling Interruption (hours)		
	DT0 n (%)	DT24 n (%)	DT48 n (%)
One	11/20 (55)	13/20 (65)	11/20 (55)
Two	7/20 (35)	7/20 (35)	9/20 (45)
Total	18/20 (90)	20/20 (100)	20/20 (100)

Table 2. Pregnancy rates by number of mating events in Santa Inês ewes following exposure to the male effect without suckling interruption (DT0), suckling interruption for 24 hours (DT24), or suckling interruption for 48 hours (DT48) during a breeding season of 45 days

Pregnancy per mating	Suckling Interruption (hours)		
	DT0 n (%)	DT24 n (%)	DT48 n (%)
First	3/11 (27.3) ab	6/13 (46.7) a	0/11 (0.0) b
Second	4/7 (57.1) b	6/7 (85.7) ab	9/9 (100.0) a
Total	7/20 (35.0)	12/20 (60.0)	9/20 (45.0)

Different letters in the same column denote statistical differences among treatments ($P < 0.05$).

The intervals between delivery and conception were 69.7 ± 6.7 days (DT0), 66.2 ± 8.8 days (DT24) and 67.8 ± 8.7 days (DT48), while the intervals between deliveries were 150.9 ± 28.6 days (DT0), 156.2 ± 29.7 days (DT24), and 149.6 ± 29.4 days (DT48) and did not differ between the groups. Prolificacy was similar among the groups, being 1.43 (DT0), 1.17 (DT24), and 1.22 (DT48).

DISCUSSION

The high percentage of cycling females was not dependent upon sucking interruption, and the male effect was the sole factor that stimulated the reproductive activity of the postpartum ewes. This result may have been found because the females were between 45 and 60 days postpartum, and as reported by GEYTENBEEK *et al.* (1984), the male effect becomes more effective in sheep when females are exposed to a male 43 days after lambing. Several reports have described that the male effect induces a preovulatory LH peak that further stimulates follicular development, leading to ovulation (CHEMINEAU, 1987; MARTIN *et al.*, 1986; PEARCE and OLDHAM, 1988; COHEN-TANNOUJJI *et al.*, 1989; DELGADILLO, 2011; LOPEZ-SEBASTIÁN *et al.*, 2014; CALDAS *et al.*, 2015).

The association of the male effect and temporary suckling interruption did not increase estrous incidence, a result that differs from previous reports that found temporary suckling interruption effective for estrous induction in goats (FALCÃO *et al.*, 2008) and beef cattle (DODE *et al.*, 1987). In addition to the male effect, temporary suckling interruption also allows an increase of GnRH and LH pulse frequency that leads to ovulation with short estrous, as reported by SHIVELY and WILLIAMS (1989).

The working hypothesis for short estrous is controversial. JAINUDEEN *et al.* (2004) argue that premature regression of the *corpus luteum* is the main cause, while CHEMINEAU *et al.* (2006) suggest the formation of the *corpus luteum* from follicles of low quality, based on a small number of luteal cells and insufficient progesterone (P4) secretion. LASSUED *et al.* (1997) described P4 deficiency as the cause of short estrous. The females described here were scored as non-cycling when they showed a P4 blood concentration lower than 1 ng/mL, as previously described by MORALES *et al.* (2003).

The male effect was capable of concentrating most estrous events within the first ten days of the breeding season. This fact is in accordance with previous data in goats and sheep (ALVES *et al.*, 2014; CALDAS *et al.*, 2015), as described by CUSHWA *et al.* (1992) and PEARCE and OLDHAM (1988), who reported efficient estrous detection. In accordance with MARTIN *et al.* (1986), two peaks of estrous detection were observed when males were introduced into the ewe herd, which was in seasonal anestrous. The first peak was observed 17-20 days after male exposure and the second at 23-26 days. Working with postpartum Santa Inês ewes, LIMA (2006) described that the first estrous was observed between the 17th

and 25th days after male exposure. As described here, most estrous events were detected between the 1st and 10th days after male introduction, while the second peak was observed between the 21st and 25th days (repeated estrous). However, the male effect did not synchronize the estrous within the first five days, as previously described by MARTIN *et al.* (1986), AZEVEDO *et al.* (1999), THIMONIER *et al.* (2000), and LIMA (2006).

The pregnancy rates described here were not considered very promising because they were below that which is expected under similar conditions for hair sheep under tropical conditions in northeast Brazil (EMBRAPA, 1994). The results are in accordance with HULET and SHELTON (2004), who stated that ewes raised under tropical conditions have lower conception rates due to several factors, such as nutrition and temperature. The findings described in our study were lower than similar experiments performed in different environments by SILVA *et al.* (1987), WILDEUS (1997) and KNIGHTS (2001). More recent research has described in more detail the importance of nutrition on male effect usage in small ruminants (SCARAMUZZI *et al.*, 2008; FITZ-RODRÍGUEZ *et al.*, 2009; URRUTIA-MORALES *et al.*, 2012).

The level of stress to which an animal is exposed affects its performance and reproductive efficiency (REALE *et al.*, 2000; LIMA 2006). The overall low pregnancy rates described here are probably due to nutritional stress during the dry period, as previously observed (SILVA *et al.*, 1987; SCARAMUZZI *et al.*, 2008; FITZ-RODRÍGUEZ *et al.*, 2009; URRUTIA-MORALES *et al.*, 2012). ERHNET and MOBERG (1991) reported that stress can affect female reproduction by lowering estrous incidence and/or conception rates. DOBSON and SMITH (1995) described that stress negatively affects reproduction through the hypothalamus by blocking LH pulses at the GnRH release level.

An important finding that correlates stress and conception failure, noted by ERHNET and MOBERG (1991), is the low pregnancy rates of the first breeding event, particularly in the DT48 females, where the longer suckling interruption increased the stress rates on the ewes. In addition to stress, several experimental evidence point to a correlation between final P4 production and fertility (OLDHAM and PEARCE, 1988; FABRE-NYS and MARTIN, 1991; CARATY and SKINNER, 1999), for example, reduced fertility after first ovulation of ewes exposed to rams, as reported by SKINNER *et al.* (2000). These authors mentioned that P4 provoked a delay in the preovulatory LH peak and affected final oocyte maturation or further follicle luteinization.

The extension of the anestrus postpartum period is one of the major limiting factors that decreases reproductive performance in livestock. The duration of postpartum anestrus is dependent upon female nutrition (GONZALEZ STAGNARO, 1991; Stagg *et al.*, 1998), breeding season period (HULET and SHELTON, 1982), male presence (GEYTENBEEK *et al.*, 1984; Lassoued *et al.*, 2004), suckling regimen (Gonzalez-Stagnaro, 1991; Falcão *et al.*, 2008) and sucking stimulus (STAGG *et al.*, 1998). The reproductive performance rates described in our study are satisfactory when compared to previous data at this location (EMBRAPA, 1994).

CONCLUSION

In conclusion, the male effect is an effective reproductive tool for short breeding seasons of postpartum ewes. However, the association of the male effect with temporary suckling interruption is not recommended under dry weather conditions as described here, possibly due to less favorable nutritional conditions.

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