

# REPRODUCTIVE OUTCOMES USING DIFFERENT DISTANCES FOR PRECONDITIONING FOR THE MALE EFFECT IN ANGLO NUBIAN GOATS DURING TWO DISTINCT CLIMATE SEASONS<sup>1</sup>

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**ABSTRACT:** The objective of the present study was to determine if separation distance between bucks and does during two distinct climate seasons could affect the reproductive performance of goats subjected to a 45-day mating season (MS). Anglo Nubian does (n = 120) were kept apart from bucks at distances of 2 m (T1), 300 m (T2), and 2000 m (T3) for 60 days prior to the 45-day MS during two distinct climate seasons [dry season (DS, February to March) and rainy season (RS, September to October)] in Sertânia, Pernambuco state, Brazil. There were no effects of distance of separation between bucks and does in any response variable evaluated. However, during the DS, the mean of the first estrous manifestation varied significantly ( $P>0.05$ ) between groups [7.13±4.49 (T1), 8.84±5.64 (T2), and 6.37±4.21 (T3) days] and during the RS [7.33±5.74 (T1), 6.60±4.88 (T2) and 8.10±4.87 (T3) days]. Similar ( $P>0.05$ ) estrous induction rates were found during both the DS [100.00% (T1), 100.00% (T2) and 95.50% (T3)] and the RS [100.00% (T1), 100.00% (T2) and 100.00% (T3)]. The estrous synchronization rate was found to be lower during the DS [36.60%; 30.00% (T1), 35.00% (T2) and 45.00% (T3)] than during the RS [56.60%; 50.00% (T1), 60.00% (T2) and 60.00% (T3)]. Pregnancy rates during the DS [ $P>0.05$ ; 80.00% (T1), 70.00% (T2) and 75.00% (T3)] were lower than during the RS [ $P>0.05$ ; 90.00% (T1), 90.00% (T2) and 95.00% (T3)]. In summary, the separation distance between bucks and does did not affect the reproductive outcome of Anglo Nubian goats over a 45-day MS under tropical conditions. Greater reproductive outcome was observed during the RS than the DS regardless of the separation distance between bucks and does.

Keywords: bioestimulation, goat, small ruminants.

## DESEMPENHO REPRODUTIVO USANDO DISTÂNCIAS DIFERENTES PARA O PRÉ-CONDICIONAMENTO DO EFEITO MACHO EM CAPRINOS DURANTE DOIS PERÍODOS CLIMÁTICOS DISTINTOS

**RESUMO:** O objetivo do presente estudo foi determinar se a distância de isolamento entre machos e fêmeas avaliado durante dois períodos climáticos distintos afeta o desempenho reprodutivo de caprinos submetidos a uma estação de monta (EM) de 45 dias. Fêmeas Anglo Nubianas (n = 120) foram afastadas dos machos por distâncias de 2 m (T1), 300 m (T2) e 2000 m (T3) por 60 dias antes da EM de 45 dias sob diferentes condições climáticas [estação seca (ES, Fevereiro a Março) e estação chuvosa (EC, Setembro a Outubro)] em Sertânia, estado de Pernambuco, Brasil. Não houve efeito da distância de pré-condicionamento entre machos e fêmeas em nenhuma variável avaliada. No entanto, durante a ES, a média da primeira manifestação de estro variou ( $P>0,05$ ) entre os grupos [7,13±4,49 (T1), 8,84±5,64 (T2) e 6,37±4,21 (T3) dias] e durante a EC [7,33±5,74 (T1), 6,60±4,88 (T2) e 8,10±4,87 (T3)]. A taxa de indução de estro foi semelhante ( $P>0,05$ ) entre ES [100,00% (T1), 100,00% (T2) e 95,50% (T3)] e EC [100,00% (T1), 100,00% (T2) e 100,00% (T3)]. A sincronização do estro foi inferior durante a ES [36,60%, 30,00% (T1), 35,00% (T2) e 45,00% (T3)] que durante a EC [56,60%,

50,00% (T1), 60,00% (T2) e 60,00% (T3)]. As taxas de prenhez na ES [P>0,05; 80,00% (T1), 70,00% (T2) e 75,00% (T3)] foram menores do que em EC [P>0,05; 90,00% (T1), 90,00% (T2) e 95,00% (T3)]. Em conclusão, a distância de pré-condicionamento entre machos e fêmeas não afetou o desempenho reprodutivo durante a EM de 45 dias de caprinos Anglo Nubianos sob condições tropicais. Um desempenho reprodutivo maior foi observado durante a estação chuvosa comparado a estação seca, independente da distância de pré-condicionamento.

Palavras-chave: bioestimulação, caprinos, pequenos ruminantes.

## INTRODUCTION

Goat production is of significant economic and social relevance throughout the world. However, goat farms, particularly in Brazil, are frequently characterized by low levels of organization, an absence of zootechnic records, and rudimentary livestock management practices (MEDEIROS *et al.*, 1994). These characteristics yield low productivity indexes and limited overall competitiveness (SIMPLÍCIO *et al.*, 2002). As such, new approaches that improve goat production for most producers at lower cost are needed.

The “male effect” is a simple strategy of reproductive management that stimulates and synchronizes estrous cycles in goats and sheep (DELGADILLO *et al.*, 2006; HAWKEN and MARTIN, 2012; KELLER and LÉVY, 2012). This is an important alternative to using hormone-based protocols, which, although viable from a technical standpoint, require relatively expensive pharmacological products and conflict with the growing public demand for hormone-free livestock production (DELGADILLO *et al.*, 2006). In consequence, the male effect is being revisited and further explored for widespread application in goat and sheep production.

Despite intense research on the male effect, the minimal conditions to achieve its full potential with regard to females’ reproductive behavior and performance remain a matter of debate (DELGADILLO *et al.*, 2006; HAWKEN and MARTIN, 2012; KELLER and LÉVY, 2012). With regard to the preconditioning period, CHEMINEAU (1983) suggested that 21 to 28 days is sufficient, while other researchers have concluded that preconditioning requires two weeks (MARTIN *et al.*, 1986). In practice, producers often opt to isolate the animals for a few weeks (ROSA and BRYANT, 2002). In terms of preconditioning distance between males and females, PEARCE and OLDHAM (1988) recommend that rams be kept at a minimum one kilometer from the ewe herd and should not be able to see, hear or smell them. However, other researchers have questioned this conclusion

(DELGADILLO *et al.*, 2006). Because the majority of studies on the male effect have focused on sheep under marked photoperiod conditions (UNGERFELD, 2003), less is known about the preconditioning requirements for the male effect in goats, particularly under tropical conditions.

The objective of the present research was to evaluate the distance between males and females during the preconditioning period on goat estrous behavior and pregnancy rate during mating seasons of 45 days under two weather conditions (dry and rainy).

## MATERIALS AND METHODS

### Experimental Location

The research was carried out in Sertânia, Pernambuco state, Brazil (8°05' S, 37°16' W), at an altitude of 558 m. The average annual temperature at this location is 25°C and average annual rainfall is 431 mm<sup>3</sup>, from February to June, with most rainfall being concentrated in March and April.

### Animals

Three bucks with proven fertility were selected and maintained in individual pens during the preconditioning period. During the experiment, the bucks were kept near to the females in a semi-extensive system, being released in the morning into pickets formed by native pastures and returned to the fold at the end of the afternoon. A total of 120 Anglo-Nubian females ranging in age from 2 to 5 years were selected. Sanitary procedures included removal of manure from the fold, systematic deworming, and rabies and clostridial vaccines.

### Breeding season and animal nutrition

Two 45-day mating seasons (MS) occur annually, one during the rainy season (RS, from February to March), and again during the dry season (DS,

from September to October). In both periods, the food supplied to bucks prior to the initiation of the experiment consisted of elephant grass (*Pennisetum purpureum*, Schum.) offered in a fold, a 200 g concentrate specially designed for goats (Durancho®), and 200 g of corn grain (*Zea mays*, L) per animal, as well as mineral salt and water *ad libitum*. During the breeding seasons, bucks and females were fed with native pasture, which consisted of marmeleiro (*Cynodia vulgaris*, L.), jurema preta (*Mimosa nigra*, Hub.), hard nut brittle (*Cordia leucocephala*, Moric.), mororó (*Bauhinia cheilanta*, Steud.), embira jurema (*Pithecolobium diversifolium*, Benth.), and cultivated pasture (*Cenchrus ciliaris*, L.), as well as mineral salt and water *ad libitum*. During the DS, animals were also supplemented with grass silage (*Pennisetum purpureum*, Schum.).

### Preconditioning distance

Sixty days before the start of the MS, breeding males were separated from the females in accordance with each treatment (T). Individuals in group T1 were kept at a distance of 2 m from females but within visual, hearing, and olfactive range, whereas males in groups T2 and T3 were separated from the females at distances of 300 m and 2000 m, respectively (i.e., out of visual, hearing, and olfactive range).

Bucks were subjected to a breeding soundness evaluation (testicular consistency, semen concentration, vigor, motility, major and minor spermatozoa defects) the day prior to the onset of the MS in order to assess their reproductive capacity, as recommended by CBRA (2013). Bucks were introduced to the flock at a male-to-female ratio of 1:20 (i.e., a total of 3 bucks to a total of 60 does in each weather season) and were anointed with a mixture of grease and check dye (at a ratio of 4:1) in the breastbone region in order to identify females in heat. This dye was changed every ten days to facilitate identification for the females, which were coming back into estrous. Females were evaluated and given a body-condition score on the same day, using methodology recommended by GONZALEZ-STAGNARO (1991). They were also weighed and tagged with individually numbered plastic ear tags.

### Progesterone sampling and pregnancy diagnosis

The reproductive status of all ewes was assessed at 60 and 80 days following partition via ultrasound

and vaginoscopic examinations and serum progesterone dosage (SANTOS *et al.*, 2004; GRUNERT *et al.*, 2005ab). Females were considered cycling when progesterone levels were  $\geq 1$  ng/mL (MORALES *et al.*, 2003). Blood samples for progesterone dosage were collected in vacutainer tubes after puncturing the jugular vein. The serum was transferred to 0.75 mL polypropylene tubes and stored at  $-20^{\circ}\text{C}$ . Progesterone concentration was determined by chemiluminescence.

Trained personnel observed the flocks for 60 min twice daily (at 6:00 h and 16:00 h) and recorded any mating events. Estrous were recorded as induced when observed until day 5 after the onset of the MS. Diagnosis of pregnancy was performed by transrectal ultrasonography 60 days after last mating, as previously described by SANTOS *et al.* (2004).

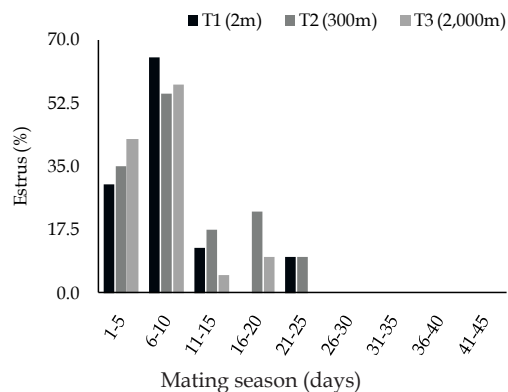
### Statistical analysis

Results were analyzed using descriptive statistics. ANOVA and Tukey's test were used to compare averages, while a Chi-square test and Fisher's exact test were used to compare proportions. All data were evaluated for normal distribution. The level of significance was 5%.

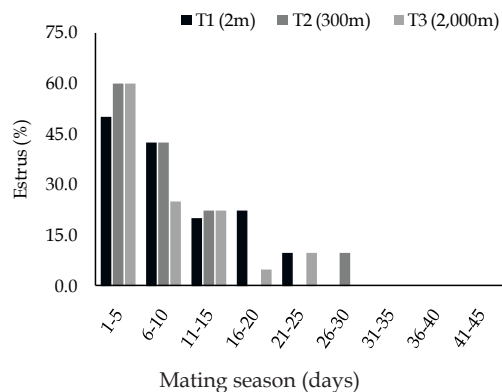
## RESULTS

Estrous behavior was predominantly observed from the 1st to the 10th day of the mating season, during both the DS (Figure 1) and the RS (Figure 2). During the DS, the mean period for first sign of estrous was  $7.13 \pm 4.49$ ,  $8.84 \pm 5.64$ , and  $6.37 \pm 4.21$  days for T1, T2 and T3, respectively, while during the RS, the mean period for first sign of estrous was  $7.33 \pm 5.74$ ,  $6.60 \pm 4.88$  and  $8.10 \pm 4.87$  days for T1, T2 and T3, respectively. Moreover, the total average value for the first sign of estrous was similar ( $P > 0.05$ ) between the two seasons (DS:  $7.43 \pm 4.82$  days, RS:  $7.34 \pm 5.13$  days).

Estrous behavior within the first five days of the MS during the DS was observed in 36.60% of all females, regardless of the treatment [30.00% T1, 35.00% T2, and 45.00% T3 ( $P > 0.05$ )]. During the first five days of the RS mating season, estrous behavior occurred in 56.60% of all females, regardless of the treatment [50.00% T1, 60.00% T2 and 60.00% T3 ( $P > 0.05$ ; Figure 2)].



**Figure 1.** Distribution of estrous in female Anglo Nubian goats kept at a distance of 2 m (T1), 300 m (T2) and 2000 m (T3) from bucks before a 45-day mating season during the dry season.



**Figure 2.** Distribution of estrous in female Anglo Nubian goats kept at a distance of 2 m (T1), 300 m (T2) and 2000 m (T3) from bucks under different preconditioning distances of T1 (2 m), T2 (300 m) and T3 (2000 m) before a breeding season of 45 days during the rainy season.

Table 1 shows the data concerning the occurrence of single estrous and of the shortened estrous cycle (<17 days) of repeated estrus. There was no significant difference ( $P>0.05$ ) detected among treatments during different estrous periods or between the DS and the RS. The average of P4 concentration and cycling rate of females during the DS and the RS are shown in Table 2.

Data related to pregnancy in the first and second services, as well as prolificacy from all treatments in the DS and RS, are shown in Table 3. There was no difference between the two seasons with regard to the number of female pregnancies in the first or second service related to treatment ( $P>0.05$ ). Additionally, there was no effect of treatment on prolificacy (Table 3).

**Table 1.** Percentages of single estrous and repeated estrous within the short estrous cycle (<17 days) and the normal cycle (17–25 days) of female Anglo Nubian goats separated from bucks by 2 m (T1), 300 m (T2) and 2000 m (T3) before a 45-day mating season, in both dry and rainy seasons

Estrous Type	Dry Season				Rainy Season			
	T1 n (%)	T2 n (%)	T3 n (%)	Total	T1 n (%)	T2 n (%)	T3 n (%)	Total
Single estrous	12 (60.0)	11 (55.0)	14 (70.0)	37 (61.6)	11 (55.0)	12 (60.0)	13 (65.0)	36 (60.0)
Short cycle	8 (40.0)	8 (40.0)	5 (25.0)	21 (35.0)	8 (40.0)	6 (30.0)	7 (35.0)	21 (35.0)
Normal cycle	0 (0.0)	1 (5.0)	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.0)	0 (0.0)	3 (15.0)
Total	20 (100.0)	20 (100.0)	19 (95.0)	59 (98.3)	20 (100.0)	20 (100.0)	20 (100.0)	60 (100.0)

No significant difference was observed ( $P>0.05$ ).

**Table 2. Percentages of cycling female Anglo Nubian goats, in agreement with serum progesterone concentration (P4), in both dry and rainy seasons**

	Treatment	Number of animals	P4 (ng/mL)	Cycling n (%)
Dry Season	T1	20	0.82±0.44 A	5 (25.0) A
	T2	20	0.75±0.53 A	4 (20.0) A
	T3	20	0.66±0.52 A	4 (20.0) A
Rainy Season	T1	20	1.58±0.50 B	16 (80.0) B
	T2	20	1.31±0.79 B	14 (70.0) B
	T3	20	1.33±0.26 B	15 (75.0) B

Different capital letters in the same column indicates a significant difference ( $P < 0.05$ ).

**Table 3. Percentage of pregnancy by service number and prolificacy in female Anglo Nubian goats separated from bucks by 2 m (T1), 300 m (T2) and 2000 m (T3) over a 45-day mating season, in both dry and rainy seasons**

Pregnancy	Dry Season				Rainy Season			
	T1 n (%)	T2 n (%)	T3 n (%)	Total	T1 n (%)	T2 n (%)	T3 n (%)	Total
First estrous	9/12 (75.0)	8/11 (72.72)	10/14 (76.92)	27/37 (72.90)	9/11 (81.81)	11/12 (91.66)	12/13 (92.3)	32/36 (88.80)
Second estrous	7/8 (87.5)	6/8 (75.0)	5/6 (83.33)	18/22 (81.80)	9/9 (100.0)	7/8 (87.5)	7/7 (100.0)	23/24 (95.80)
Total	16/20 (80.0)	14/19 (73.68)	15/20 (75.0)	45/59 (76.30)	18/20 (90.0)	18/20 (90.0)	19/20 (95.0)	55/60 (91.60)
Prolificacy	1.34	1.33	1.35	1.34	1.35	1.50	1.38	1.41

No significant difference was observed ( $P > 0.05$ ).

## DISCUSSION

It has been suggested that the male effect requires the complete isolation of males from females, in order to avoid visual, physical, hearing, or olfactory contact between the sexes (CHEMINEAU, 1983, 1987; WALKDEN-BROWN *et al.*, 1993; HAWKEN and MARTIN, 2012; KELLER and LÉVY, 2012). More recent reports have suggested that the presence of bucks or rams does not induce refractoriness to the male effect (DELGADILLO *et al.*, 2006; VÉLIZ *et al.*, 2006). The introduction of new fertile males into these herds (containing males and females) triggers physiological and behavioral phenomena similar to those observed in females previously isolated from males (DELGADILLO *et al.*, 2006; VÉLIZ *et al.*, 2006; HAWKEN and MARTIN, 2012). Despite the relevance of these findings to the understanding

of the male effect on female reproductive physiology, experimental settings are not practical to commercial goat and sheep production systems. In the present report, only the physical isolation of bucks was tested as a practical and simple criterion for the application of the male effect and to address its influence on the reproductive performance of Anglo Nubian does. However, the decision to avoid physical contact between animals inhibits some mechanisms that stimulate pheromone secretion and endocrine responses that ultimately lead to changes in reproductive behavior (VANDENBERGH, 1988; HAFEZ and HAFEZ, 2004). Moreover, it is known that reproductive activity in females is principally stimulated by androgens (FULKERSON *et al.*, 1981; SIGNORET *et al.*, 1982), by sebaceous glands stimulated by steroids (HILLBRICK and TUCKER, 1996; IWATA *et al.*, 2000) and even by animal hair

(KNIGHT and LYNCH, 1980; SIGNORET and LINDSAY, 1982). This chemical communication increases pituitary activity mediated by pheromones, triggering a neuroendocrine response that results in hypothalamic liberation of GnRH, followed by pulsatile LH release, and finally resulting in estrous and ovulation. Reproductive behavior was also stimulated by social stimuli (MARTIN *et al.*, 1986; CHEMINEAU, 1985, 1987; THOMPSON, 2006).

Despite the lack of physiological seasonality of Anglo Nubian goats under tropical conditions, most females were acyclic in DS conditions based on P4 values. Thus, the high rate of estrous synchronization in all treatments can also be attributed to active cycling conditions as a result of the male effect in all females during both the DS and the RS. The progesterone profile that was used to determine the condition of female cyclicity was similar in all treatments under the same weather conditions.

Goats commonly show signs of short estrous cycles during the mating season due to various factors, including nutrient deficiency (GONZALEZ-STAGNARO, 1991), postpartum condition (CHEMINEAU, 1983), the amount of rainfall (SIMPLÍCIO *et al.*, 1986; CERBITO *et al.*, 1995), and by the male effect (CORTEEL, 1994). In these studies, estrous incidence during both the DS and the RS was considered to be low when compared to other research where populations of acyclic goats were exposed to males (LIMA, 1998; CHEMINEAU, 1983). The lack of influence from weather conditions points to species-specific, albeit unidentified, factors causing these short estrous cycles. It would appear to be a natural tendency of this species to develop this type of cycle, as reported by CHEMINEAU (1986). Other hypotheses to describe short estrous cycles have been described. Short estrous cycles seem to be associated with a low concentration of LH, which would cause a corpus luteum deficiency and affect the ability to maintain the luteum function for a normal period of time (LIMA, 1998; SHELTON, 1980). According to JAINUDEEN *et al.* (2004), these short cycles are due to premature corpus luteum regression, or possibly due to lower-quality follicle production that ultimately leads to abnormal corpus luteum development and limited progesterone synthesis (CHEMINEAU *et al.*, 2006; LASSUED *et al.*, 1997).

Despite the results regarding estrous incidence and the changes in female behavior described above, these factors are only potential indicators for overall fertility and prolificacy following the use of the male effect. Females were examined for pregnancy outcome and kid prolificacy after

both breeding seasons; as it turns out, 90% of all pregnancies occurred in the first MS, demonstrating the potential of the male effect. Moreover, the pregnancy and prolificacy rates described here fall within the range described by other authors (LIMA, 1998; FOLCH *et al.*, 1993; CHEMINEAU, 1987). Remarkably, the weather conditions under which our study took place did not influence pregnancy or prolificacy rates.

## CONCLUSION

The distance of separation between bucks and does did not affect the reproductive outcome during the 45-day mating season of Anglo Nubian goats under tropical conditions. Greater reproductive outcome was observed during the rainy season than the dry season, regardless of the separation distance between the bucks and does.

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