The influence of separation distance during the preconditioning period of the male effect approach on reproductive performance in sheep

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Abstract

The study was aimed to test the effect of the separation distance between males and females during the preconditioning period on the reproductive performance of Santa Inês ewes after the male effect. Santa Inês ewes were kept at distances of 3000 m (T1), 3 m (T2), and 300 m (T3) from rams for 60 days before starting 45-day mating seasons during the dry period (DP) and rainy periods (RP). Mating events were observed daily at 6:00 h and 16:00 h by trained personnel for one hour intervals. Estrous were scored as synchronized when observed until day 5 after breeding season start. Pregnancy diagnosis was performed by ultrasonography. In the DP, the first estrous averaged at 15.45±10.36 (T1), 9.25±6.41 (T2) and 13.05±10.24 (T3) days and in RP was 8.73±5.84 (T1), 9.30±5.62 (T2) and 6.10±5.66 (T3) days. All females cycled during both DP and RP. Estrous synchronization occurred in 20% of the females during DP (T1: 30%, T2: 15%, and T3: 15%). In the RP, estrous synchronization occurred in 40% of all females (T1: 30%, T2: 35%, and T3: 45%). The pregnancy rates in DP and RP were T1: 85%, T2: 80%, and T3: 75%. The results show that the male effect can be obtained simply by avoiding physical contact between males and females throughout the year under tropical conditions.

Introduction

One common caveat of sheep farming is the heterogeneity of wool, milk and meat production throughout the year. Reproduction plays a major role in sheep production, where reproduction efficiency correlates with overall profitability. Control of reproduction allows production planning and practices such as estrous synchronization allows lambing at expected time intervals for maximizing profit.[1]

The introduction of a ram or buck in a female flock triggers a physiological response that ultimately leads to estrous onset in anestrous animals and synchronizes estrous in cycling females, namely ram effect or male effect.[2,3] This natural phenomenon has been extensively studied for scientific purposes, but the potential of the male effect has not been translated fully to commercial settings.

Based on earlier investigations, male effect usage has been described under two gold standard practices: preconditioning period of two to four weeks (separation of males and females)[4-6] and preconditioning distance of at least 1000 m (separation distance between males and females).[6] These parameters were established basing on the concept that the male effect required complete isolation between genders.[3,6] However, recent work has demonstrated that physical (and consequently olfactory, auditive and hearing) isolation between genders is dispensable for male effect usage based ovulation induction in sheep and goats.[3,7] However, these studies dissected the physiological response and ovulation parameters of omitting ram physical isolation for male effect,[3,7,8] but further reproductive parameters such as pregnancy and prolificacy rates remained to be described.

The objective of the present research was to test the distance between males and females during the preconditioning period on estrous synchronization, pregnancy rates and prolificacy of cycling Santa Inês ewes during 45-day breeding seasons throughout the year.

Materials and Methods

This study was conducted in Sertânia, Pernambuco state, Brazil. Geographic coordinates are: 9.107.002 Km N and 691.005 Km E, altitude of 558 m, mean annual temperature of 25ºC, annual rainfall of 431 mm, where the rainy season is from February to June.

Three rams with proven fertility and one hundred and twenty Santa Inês multiparous ewes were selected for the project. During the experiment, females were kept in semi-extensive system and males were housed individually. Breeding seasons of 45 days were carried out from February to March (rainy period - RP), and from September to October (dry period - DP). During both seasons, rams were fed with hay (*Pennisetum purpureum*, Schum.), 200 g per day of grain concentrate for sheep (Durancho®, Pesqueira, Brazil). During the breeding season, all animals were fed with native pastures or cultivated pastures (*Cenchrus ciliaris*, L.). During the DP, ewes received hay silage supplementation (*Pennisetum purpureum*, Schum.). All animals had free access to mineral salt and water throughout the experiments.

Sixty days before beginning the breeding season, rams were separated from ewes, according to each experimental group: T1: 3000 m, T2: 3 m, and T3: 300 m, where T1 and T3 distances allowed no physical, visual, auditive, or olfactive contact. In contrast, T2 avoided physical contact between animals only.

The day before breeding season onset, ram were submitted to andrologic examination to confirm their fertility status.[9] Rams were introduced in the ewe herd in an 1:20 male to female ratio. Rams were marked every ten days with wax and ink (4:1) on the sternum

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region to further identify females after mating (cycling females). All females were evaluated for body condition score,[10] weighted, and properly identified with ear tags.

The reproductive status of all ewes was assessed by ultrasonographic and vaginoscopic examinations and progesterone dosage 60 and 80 days after partuition.[11,12] Females were considered cycling when progesterone (P4) levels reached 1 ng mL^{-1}.[13] Blood samples for progesterone dosage were collected in vacutainer tubes by punctioning the jugular vein, transferred to 0.75 mL polypropylene tubes, and stored at –20°C until further use. P4 level was quantified by chemiluminescence. Based on ultrasonographic exam and P4 dosage, only cycling non-lactating females were further used in the experiment. Mating events were observed daily at 6:00 h and 16:00 h by trained personnel for one hour intervals. Estrous were scored as synchronized when observed until day 5 after breeding season start. Pregnancy diagnosis was performed by transrectal ultrasonography on day 60 after the last mating.[11]

Results were analyzed by ANOVA, by Kruskal-Wallis test, and Exact Fisher's test. The Statistical Analysis System (SAS) software was used for running the analysis. An 5% difference between groups was considered significant.

Results

Estrous manifestation was observed throughout both breeding seasons, being most frequently observed within day 1 and 10, both during the DP (Figure 1A) and RP (Figure 1B).

The first estrous during the DP was observed between day 1 and 32 of the breeding season, and during the RP, within day 1 and 23. During the DP, mean value of the first estrous was 15.45±10.36 (T1), 9.25±6.41 (T2), and 13.05±10.24 (T3) days, and RP was 8.73±5.84 (T1), 9.30±5.62 (T2), and 6.10±5.66 (T3) days. Moreover, it can be observed that estrous synchronization within the first five days of the breeding season during the DP reached 20% of all females, where 30% were from the T1, 15% from T2, and 15% from T3 (P>0.05; Table 1, Figure 1).

During the RP, 37% of all females were identified in estrous during the initial five days of the breeding season, where 30% were from T1, 35% from T2, and 45% from T3 (P>0.05). The mean value for the first estrous manifestation during the DP was 7.95±5.44 days, and during the RP was 12.58±9.40 days (P>0.05).

In order to better characterize the type and frequency of estrous being detected, estrous events were classified as short estrous cycles (<11 days) or as regular cycles (≥11 days). All females cycled during the 45 day breeding season during the DP, where 21 had two estrous (35%), 18 had short cycles (30%), and 21 had one regular cycle (35%; P>0.05). During the RP, all 60 females cycled, where 15 cycled twice (25%), 36 had short estrous cycles (60%), and 9 had one regular cycle (15%).

In order to address the viability of estrous induction and resulting ovulations, cycling females were allowed to mate to fertile rams during the breeding season. The data describes the pregnancy rates after first and second mattings, as well as prolificacy from all treatments on DP and RP breeding seasons (Table 2). During both DP and RP, pregnancy rates were similar across groups and varied from 75% to 85% (P<0.05). Regarding the prolificacy data, it was observed that on DP it varied from 1.13 to 1.38 and 1.12 to 1.26, on the DP and RP, respectively.

Discussion and Conclusions

It has been claimed that one of the main requirements for the male effect is the complete isolation of males from females during three to four week periods.[4,6,14-16] However, more recent reports have question the necessity of physical isolation between genders for the male effect.[3,7] Moreover, to our knowledge, this recent discovery has not been tested under commercial settings, and it remained unexplored if pregnancy and prolificacy rates are

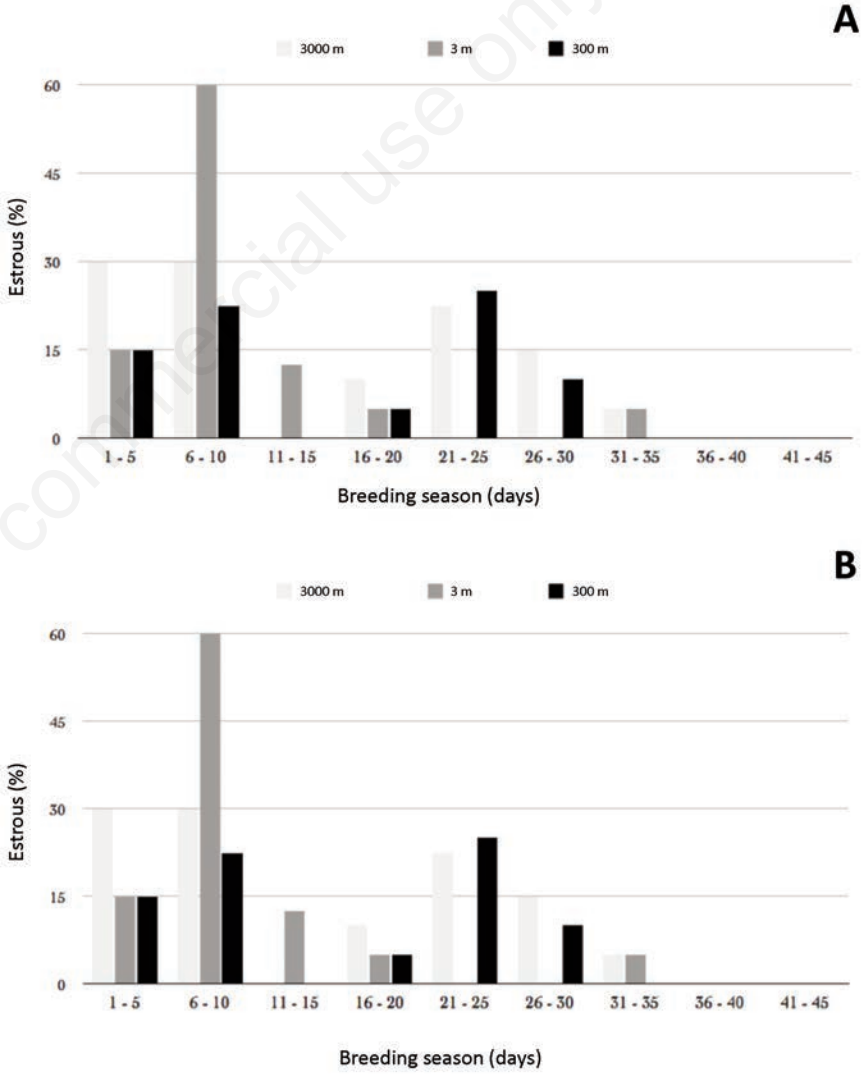

Figure 1. Estrous distribution in Santa Inês ewes preconditioned for male effect under 3000 m (T1), 3 m (T2) and 300 m (T3) distances before an 45 day breeding season during the dry (A) and rainy (B) season.

Table 1. Percentage of double estrous, short simple estrous (<11 days), normal single estrous (≥11 days) of Santa Inês ewes preconditioned for male effect under 3000 m (T1), 3 m (T2) and 300 m (T3) during 45 day breeding seasons under different weather conditions.

Estrous type	Dry				Rainy			
	T1 n (%)	T2 n (%)	T3 n (%)	Total n (%)	T1 n (%)	T2 n (%)	T3 n (%)	Total n (%)
Two estrous	7/20 (35.0)	8/20 (40.0)	6/20 (30.0)	21/60 (35.0)	4/20 (20.0)	4/20 (20.0)	6/20 (30.0)	14/60 (23.3)
Single short estrous	2/20 (10.0)	11/20 (55.0)	5/20 (25.0)	18/60 (30.0)	15/20 (75.0)	dic-20 (60.0)	ott-20 (50.0)	37/60 (62.7)
Single estrous	11/20 (55.0)	1/20 (05.0)	24/20 (45.0)	21/60 (35.0)	1/20 (05.0)	4/20 (20.0)	4/20 (20.0)	6/60 (15.0)
Total	20	20	20	60	20	20	20	60

Table 2. Pregnancy rates and prolificacy per number of services of Santa Inês ewes preconditioned for male effect under 3000 m (T1), 3 m (T2), and 300 m (T3) during 45 day breeding seasons under different weather conditions.

Estrous type	Dry				Rainy			
	T1 N (%)	T2 n (%)	T3 n (%)	Total n (%)	T1 n (%)	T2 n (%)	T3 n (%)	Total n (%)
First Maitimg	12/13 (92.3)	12/09 (75.0)	11/14 (78.6)	32/39 (82.0)	13/16 (81.3)	12/16 (75.0)	10/14 (71.4)	35/46 (76.1)
Second Mating	7/05 (71.4)	8/07 (87.5)	6/04 (66.7)	16/21 (76.2)	4/04 (100.0)	4/04 (100.0)	6/05 (83.3)	13/14 (93.0)
Total	17/20 (85.0)	16/20 (80.0)	15/20 (75.0)	48/60 (80.0)	17/20 (85.0)	16/20 (80.0)	15/20 (75.0)	48/60 (80.0)
Prolificacy	1.29	1.38	1.13	1.27	1.12	1.25	1.26	1.21

affected when ram isolation is omitted.

Small ruminant production under tropical conditions offers the advantage that animals do not show seasonal anestrous. Under these circumstances, the male effect can be used throughout the year as a simple procedure to synchronize estrous and ultimately concentrate births within short periods of time. Despite these advantages, other factors such as food availability may affect the reproductive performance in sheep. In the present report, biostimulation was more effective during the RP, probably due to the higher quality and quantity of native pastures available.[17,18] Stress affects reproduction, influencing the hypothalamus and blocking luteinizing hormone release, which may ultimately lead to anestrous or diminished estrous behaviour.[19] The incidence of short cycles was considered high, specially during the RP. This data is counterintuitive, because short cycles were expected to be more frequent during the DP, or in animals under anestrous conditions. Further research is needed to address possible causes for these results.

The pregnancy rates described here, both on DP and RP, were under the expected fertility rates observed under Brazilian tropical semi-arid conditions.[20,21] However, these numbers are different than other reports, which found that sheep raised under tropical conditions are affected by factors such as nutrition and temperature on conception rates.[22] The data described here for conception rate is similar to reports by other authors under temperate conditions during the breeding season, and superior to other breeds: St. Croix (55%), Polyplay (50%), Dorset (68%), Santa Inês (42%), native (65%).[17,23] The prolificacy data was similar between DP and RP. This data is in agreement with previous reports that could not find any influence of weather conditions on ewes prolificacy.[24]

In conclusion, ram isolation from females before the breeding season is dispensable for male effect under tropical conditions, during both DP and RP, since it does not influence the reproductive performance of Santa Inês ewes.

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