Male effect associated to breeding season shortening impacts upon reproductive performance of cycling pluriparous ewes under different climate conditions

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Abstract: It was aimed to evaluate the influence of male effect and breeding season shortening upon estrus induction and synchronization, pregnancy rate and prolificacy of cycling pluriparous ewes of Santa Inês (n = 180) and Morada Nova (n = 180) breeds under both semiarid and subtropical semi humid conditions. Females were selected based upon body condition score and ciclicity status, and further isolated from males for 30 days. Rams of Santa Inês (n = 6) and Morada Nova (n = 6) were pre-selected based upon fertility records and further tested by andrology test on the before experiment onset. Under Semiarid conditions, the percentage of females displaying estrus for Santa Inês ewes varied from 90.0% to 96.6% and 90.0% to 93.3% for Morada Nova ewes, with no difference between breeds ($P > 0.05$). In the tropical semi humid region, the percentage of Santa Inês females displaying estrus was 100% and for Morada Nova ewes it varied from 93.3 to 100%, with no difference between breeds ($P > 0.05$). Comparisons between regions did not display any difference within or between breeds ($P > 0.05$). In the semiarid, irrespectively of the breed, 167/180 (92.7%) females displayed estrus until day 40 of the breeding season and estrus synchronization occurred in 55.0% of Santa Inês ewes and 63.0% Morada Nova ewes. In tropical semi humid, irrespectively of the breed, 177/180 (98.3%) females displayed estrus until day 40 and estrus synchronization in 60.0% Santa Inês females and 64.0% Morada Nova females. In semiarid, pregnancy rate for Santa Inês females varied from 85.2% to 96.3% and 85.7% to 92.8% for Morada Nova females ($P > 0.05$). In tropical semi humid, pregnancy rates were similar for Santa Inês females (86.6% to 90.0%) and 90.0% to 93.1% for Morada Nova females ($P > 0.05$). In semiarid, irrespectively of breed, the prolificacy varied from 1.29 ± 0.45 to 1.35 ± 0.47 and 1.35± 0.47 to 1.42 ± 0.49 for tropical semi humid, with no difference between breeds and within regions. In conclusion, male effect associated to breeding season duration shortening to 35 days is efficient to induce and synchronize estrous in cycling pluriparous ewes while maintaining pregnancy and prolificacy rates.
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1 – Introduction

Sheep production is an socioeconomically activity throughout the world, as an alternative source of meat, milk, skin and wool. Furthermore, the gap between the socioeconomic value and productivity of sheep production systems has been minimized with the adoption of technologies that increase genetic gain per generation while increasing reproductive performance in herds (Mazzoni Gonzalez and Oliveira, 1991). Within these technologies, estrous cycle control, by natural approaches (Almeida-Irmão et al., 2014; Alves et al., 2014, 2015 and Caldas et al., 2015) or chemically-based approaches (Menchaca et al., 2004; Rubianes et al., 2004; Uribe-Velásquez et al., 2010, 2011) has been used in artificial insemination and embryo transfer programs (Killen and Caffery 1982; Luz et al., 2000 and Fonseca, 2007).

Reproductive management control by simple practices are indispensable for profitability in sheep production. In this context, male effect is a social stimulus (Fraser, 1980 and Ramirez et al., 2001) that

has the potential to induce and synchronize estrus at low cost. The biostimulation by the male effect is through chemical interaction mediated by pheromones that promote endocrine responses that ultemetely lead to reproductive behavior changes in animal at intraspecific and interspecific fashions (Over et al., 1990; Rekwot et al., 2001). The male effect holds the advantage of anticipating mating events during the breeding season or to increase the synchronization of deliveries and weaning in the herd (Martin et al., 1986 and Evans et al., 2004). Use of bioestimulation associated with breeding season usage is directly related to an increase in reproductive performance of cycling pluriparous ewes (Lima, 2006 and Caldas et al., 2015).

It was aimed to evaluate the influence of male effect associated with breeding season duration on estrous induction and synchronization, pregnancy rates and prolificacy of cycling pluriparous Santa Inês and Morada Nova ewes raised under semiarid and tropical semi humid conditions.

2 - Material and Methods

The experiment was conducted in Pernambuco state, under semiarid conditions, from August through September, and tropical semi humid conditions from October through November. The geographic coordenates of Sertânia in the Semiarid region are latitude 08° 04' 25" S, longitude 37° 15' 52" O, altitude of 558 m, mean annual temperature of 22.8 °C and mean annual rainfall of 566 mm^3. The climate is semiarid, with rainfall from February to June. O Município de Escada, situado na Região da Zona da Mata Sul, apresenta como coordenadas geográficas, latitude 08° 21' 33" S, longitude 35° 13' 25" O, altitude of 109 m, mean annual temperature of 24.4 °C and mean annual rainfall of 1763 mm^3. The climate is tropical semi humid, with rainy period from May until August.

In the Semiarid region, animals were fed with native pastures containing *Cynodia vulgaris*, *Mimosa nigra, Hub.*, *Cordia leucocephala, Moric.*, *Bauhinia cheilanta, Steud.*, *Pithecolobium diversiffolium, Benth.,* and cultivated pastures of *Digitaria decumbes* and *Cenchrus ciliaris L.* Animals were further supplemented with silage (*Sorghum bicolor, Moench.*) and small forage cactus (*Napolea cochenillifera, Salm-Dick)* in the afternoon. Animals had free access to mineral salt and water.

In the tropical semi humid region, animals were fed with native pastures containing *Paspalum maritimum*, *Chloris orthonton*, *Cynodon dactylon* and *Brachiaria tunnergrass* or cultivated pasture of *Brachiaria humidicola* and further supplemented with hay (*Pennisetum purpureum*) during the afternoon. Animals had free access to mineral salt and water.

Females with age ranging from 36 to 48 months, of Santa Inês (n = 180) and Morada Nova (n = 180) were pre-selected after weaning their lambs and their cyclicity status (at least two estrus were detected using vasectomized males or visual appraisal by trained personnel. In order to induce the male effect, females were isolated from males for 60 days without any physical, visual, olfactive and auditive contact. The day before experiment onset, females were evaluated for body condition score, and were selected when score was between 3 (good) and 4 (excellent), using an 1-5 scale as previously described (Gonzalez-Stagnaro, 1991).

Females were identified with plastic ear tags, weighted and randomly allocated to groups under breeding seasons for different durations: 25 days (BS25), 35 days (BS35) and 45 days (BS45). Estrus were detected by visual appraisal twice a day at 6:00 and 16:00 hours, for one hour periods by trained personnel. Estrous were considered synchronized when detected within the initial five days of the breeding season.

Rams of Santa Inês (n = 6) and Morada Nova (n = 6) breeds of proven fertility were pre-selected for the experiment. On the week before experiment onset, rams were further selected based upon their reproductive potential by an andrology exam (CBRA 1998). Rams had the sternum bone region covered with a misture wax and ink (4:1), in order to ease the identification of females in estrous. The ink color was replaced within every ten days of the breeding season. Pregnancy diagnosis was performed by ultrasonography on day 60 after the last recorded mating event (Santos et al., 2004).

The statistical analysis was performed with univariate and bivariate absolute and percentage distribution with means and standard deviation and t-Student test for independent samples with same or different variances. Tua data was analyzed by ANOVA with SAS, version 8. Differences with probability of $p < 0.05$ were considered significant.

3 - Results

Irrespectively of the region, breed and breeding season duration, a high number ($P < 0.05$) of females displayed one estrous, and the number females with two estrous recorded was higher than ($P < 0.05$) the number of females with three estrous (Table 1).

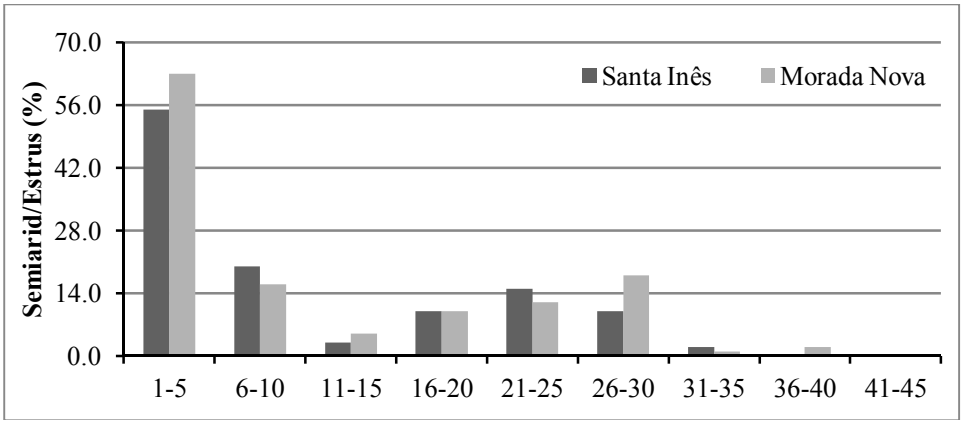
Table 1. Number of estrus detected in Santa Inês and Morada Nova cycling pluriparous ewes submitted to male effect associated to breeding seasons of 25 days (BS25), 35 days (BS35) and 45 days (BS45) in semiarid and tropical semi humid regions.

Region												
Estrus (n)	Semiarid						Tropical Semi Humid					
	Santa Inês			Morada Nova			Santa Inês			Morada Nova		
	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS45 n/n (%)	BS35 n/n (%)	EM-25d n/n (%)	EM-35d n/n (%)	EM-45d n/n (%)	EM-25d n/n (%)	EM-35d n/n (%)	EM-45d n/n (%)
One	19/30[a] (63,3)	18/30 (60,0)	19/30[a] (63,3)	21/30[a] (70,0)	19/30[a] (63,3)	22/30[a] (73,3)	20/30[a] (66,7)	19/30[a] (63,3)	20/30[a] (66,7)	20/30[a] (66,7)	22/30[a] (73,3)	21/30[a] (70,0)
Two	8/30[b] (26,7)	11/30 (36,6)	6/30[b] (20,0)	6/30[b] (20,0)	9/30[b] (30,0)	6/30[b] (20,0)	9/30[bc] (30,0)	11/30[b] (36,7)	8/30[bc] (26,6)	9/30[b] (30,0)	8/30[b] (26,7)	6/30[bc] (20,0)
Three	-	-	2/30[b] (6,7)	-	-	-	1/30[bd] (3,30)	-	2/30[bd] (6,7)	-	-	1/30[bd] (3,3)
Total	27/30 (90,0)	29/30 (96,6)	27/30 (90,0)	27/30 (90,0)	28/30 (93,3)	28/30 (93,3)	30/30 (100)	30/30 (100)	30/30 (100)	29/30 (96,7)	30/30 (100)	28/30 (93,3)

Different superscript letters (a,b,c,d) on same column denote $P < 0.05$.

In the semiarid region, 167/180 (92.7%) females displayed estrous until day 40 of the breeding season and estrous synchronization was observed in 55.0% Santa Inês ewes and 63.0% Morada Nova ewes (fig. 1). In the tropical semi humid region, 177/180 (98.3%) females had al least one estrous detected until day 40 and synchronization occurred 60.0% Santa Inês ewes and in 64.0% Morada Nova ewes (fig 1).

The percentage of estrous in Santa Inês ewes varied from 90.0 to 96.6% and in Morada Nova ewes from 90.0 to 93.3%, with no difference ($P > 0.05$) between breeds or breeding season duration (table 1). The percentage of estrous in the tropical semi humid for Santa Inês ewes was 100% and Morada Nova varied from 93.3 to 100% ($P > 0.05$) (table 1).



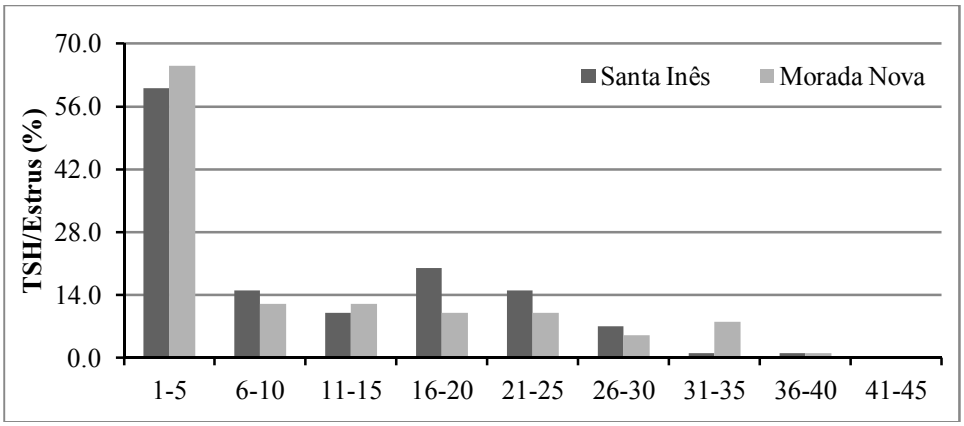


Figure 1 - Estrus distribution of cycling pluriparous Santa Inês and Morada Nova ewes submitted to male effect associated with breeding seasons of 25 days (BS25), 35 days (BS35) and 45 days (BS45) in semiarid and tropical semi humid region (TSH)

The pregnancy rate in the semiarid region was similar between breeds ($P > 0.05$), were it varied from 85.2% to 96.3% for Santa Inês ewes and 85.7% to 92.8% Morada Nova ewes. However, the pregnancy rate of Santa Inês ewes in BS35 was higher on first service ($P < 0.05$) than the second service (table 2). The pregnancy rate in the tropical semi humid varied from 86.6% to 90.0% for Santa Inês ewes and 90.0% to 93.1% for Morada Nova ewes ($P > 0.05$).

All females diagnosed as pregnant delivered singletons or twins at similar rates ($P > 0.05$) (table 3), irrespectively of breed and region. However, under both regions, the percentage of singleton delivery was higher than ($P < 0.05$) twin deliveries. Moreover, prolificacy was similar ($P < 0.05$) between breeds, breeding seasons and regions (table 3).

Table 2. Pregnancy rates of Santa Inês and Morada Nova cycling pluriparous ewes, on first, second and third services that were submitted to male effect under breeding seasons of 25 days (BS25), 35 days (BS35) and 45 days (BS45) in semiarid and tropical semi humid regions.

Regions													
	Semiarid						**Tropical Semi Humid**						
	Santa Inês			**Morada Nova**			**Santa Inês**			**Morada Nova**			
Delivery Type	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	
Single	16/2[a] (69,6)	19/2[a] (70,4)	18/2[a] (69,2)	16/2[a] (69,6)	17/2[a] (70,8)	17/2[a] (65,4)	16/26 (61,5)	17/26[a] (65,4)	17/27 (62,9)	16/27 (59,3)	16/27 (59,3)	15/26 (57,7)	
Twin	7/23[b] (30,4)	8/27[b] (29,6)	8/26[b] (30,8)	7/23[b] (30,4)	7/24[b] (29,2)	9/26[b] (34,6)	10/26 (38,5)	9/26[b] (34,6)	10/27 (37,1)	11/27 (40,7)	11/27 (40,7)	11/26 (42,3)	
T o t a l	23/23 (100)	27/27 (100)	26/26 (100)	23/23 (100)	24/24 (100)	26/26 (100)	26/26 (100)	26/26 (100)	27/27 (100)	27/27 (100)	27/27 (100)	26/26 (100)	
Prolificacy ($\overline{X}$±s)	1.30 ± 0.46	1.30 ± 0.45	1,31 ± 0,46	1,30 ± 0,46	1,29 ± 0,45	1,35 ± 0,47	1,38 ± 0,48	1,35 ± 0,47	1,47 ± 0,48	1,41 ± 0,49	1,41 ± 0,49	1,42 ± 0,49	

Different superscript letters (a,b) on same column denote $P < 0.05$.

Table 3. Delivery type and prolificacy of Santa Inês and Morada Nova cycling pluriparous ewes submitted to male effect under breeding seasons of 25 days (BS25), 35 days (BS35) and 45 days (BS45) in semiarid and tropical semi humid regions.

Regions													
	Semiarid						**Tropical Semi Humid**						
	Santa Inês			**Morada Nova**			**Santa Inês**			**Morada Nova**			
Delivery Type	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	BS25 n/n (%)	BS35 n/n (%)	BS45 n/n (%)	
Single	16/23[a] (69,6)	19/27[a] (70,4)	18/26[a] (69,2)	16/23[a] (69,6)	17/24[a] (70,8)	17/26[a] (65,4)	16/26 (61,5)	17/26[a] (65,4)	17/27 (62,9)	16/27 (59,3)	16/27 (59,3)	15/26 (57,7)	
Twin	7/23[b] (30,4)	8/27[b] (29,6)	8/26[b] (30,8)	7/23[b] (30,4)	7/24[b] (29,2)	9/26[b] (34,6)	10/26 (38,5)	9/26[b] (34,6)	10/27 (37,1)	11/27 (40,7)	11/27 (40,7)	11/26 (42,3)	
T o t a l	23/23 (100)	27/27 (100)	26/26 (100)	23/23 (100)	24/24 (100)	26/26 (100)	26/26 (100)	26/26 (100)	27/27 (100)	27/27 (100)	27/27 (100)	26/26 (100)	
Prolificacy ($\overline{X}$±s)	1.30 ± 0.46	1.30 ± 0.45	1,31 ± 0,46	1,30 ± 0,46	1,29 ± 0,45	1,35 ± 0,47	1,38 ± 0,48	1,35 ± 0,47	1,47 ± 0,48	1,41 ± 0,49	1,41 ± 0,49	1,42 ± 0,49	

Different superscript letters (a,b) on same column denote $P < 0.05$.

4 - Discussion

The application of a breeding season with a defined time frame represents one of the most important tools to increase the reproductive efficiency of a sheep herd. Estrous dispersion is naturally dependent upon the estrous cycle of each female, which represents a physiological condition that diminishes the sensibility to estradiol negative feedback (Rosa and Bryant, 2002). As described here, estrous distribution was observed until day 40 of the breeding season, where some females display two or three estrous. However, not all females were diagnosed as pregnant at the end of the breeding season. It is probable that these females carried some reproductive problem, that impedes the establishment of a pregnancy, even under an 51-day breeding season, as suggested by Eloy et al. (2001) and Guimarães Filho (2010). It is interesting to note that the number of females that had two detected estrous was lower than females with one estrous. These observations alongside with the high incidence of synchronized estrous allows the suggestion to reduce breeding season duration from 45 days to 35 days, irrespectively of breed and region, since it would reduce production costs, specially with trained personnel.

The sexual experience of pluriparous females contributes to the high receptivity of male courtship behavior and this fact leads to increased estrous onset during the breeding season (Gelez et al., 2003 ; Gelez and Fabre-Nyz, 2004). Moreover, it is common to verify a high number of females in estrous until day 15 (Caldas et al., 2015) or day 18 (Lima, 2006) after male introduction in the female flock, as described here. This fact further supports the reduction of breeding season duration, as described above, contributing to concentrate deliveries in favorable periods, resulting in more homogeneous animal lots, rationalizing nutritional and sanitary managements practices, among other advantages (Fonseca, 2010).

With the exception of Santa Inês ewes under Semiarid conditions, that were submitted to BS35, pregnancy rates were similar between first nas second services, irrespectively of region and breed, in accordance with previous reports (Caldas et al., 2015). It is possible that que these findings are related to factors that interfere in both female and male reproduction. It has been described that the first estrus after male effect usage can be anovulatory (Ungerfeld et al., 2004) or, if ovulation occurs, the corpus luteum formed is weak, lapses rapidly and ultimately leads to short estrous cycles (Hafez and Hafez, 2004; Jainudeen et al., 2004 and Chemineau et al., 2006). In males, it is relevant to mention that despite the male to female rito used was based on previous reports (Nogueira et al., 2011), and that males were submitted to andrology exams before experiment onset, estrous synchronization was observed in the majority of

females and physically demanded from males within the initial five days of the breeding season. This fact may have contributed to the higher pregnancy rate on the second service,since estrous distribution was more homogenous throughout the rest of the breeding season. Due to these facts, it can be stated that a period of 35 days should be sufficient for males to mate with all females during the breeding season. Females that are not pregnant at the end of the breeding season should be carefully evaluated and if display cyclicity should be culled aiming to increase the reproductive performance of the herd.

The results obtained with different delivery types showed significant prevalence of singleton delivery compared to twins, specially under Semiarid conditions for both breeds. Despite that these results are in agreement with previous reports (Vinagre et al., 1992; Pereira et al., 1998 and Mexia et al., 2004), it was hypothesized that tropical semi humid conditions would display similar results. However, contrary to data on Semiarid and with the exception of Santa Inês ewes under BS35, singleton and twin deliveries were recorded at similar efficiencies. This fact be related to nutritional issues, due to more abundant and better distributed rainfall that maintains pastures with better nutritional value. Tropical semi humid areas display less luminosity during raining days compared to the semiarid, during the period of a few months before experiment onset, that may have influenced positively on prolificacy, that is intimately related to circadian melatonin production. These finding on prolificacy, despite similarities between Tropical semi humid and semiarid conditions, these efficiencies are similar to previously reported for Santa Inês and Morada Nova ewes (Machado et al., 1999).

The results described here allow the conclusion that the male effect associated to shortening of breeding season duration is efficient to induce and synchronize estrous of pluriparous ewes without affecting pregnancy rates and overall prolificacy. These results collectively allow the reduction of breeding season duration from 45 to 35 days leading to lambing at shorter time frames with the same reproductive efficiency.

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