Acta Scientiarum



http://periodicos.uem.br/ojs/acta ISSN on-line: 1807-8672 Doi: 10.4025/actascianimsci.v45i1.57518

# Reproductive behavior of Pantaneiros sheep, Mato Grosso do Sul, Brazil

Charles Ferreira Martins<sup>1</sup>, Arnaldo Santiago Filho<sup>2</sup>, Marcos Barbosa Ferreira<sup>3</sup>, Carlos Eurico dos Santos Fernandes<sup>4</sup>, Antonio Carlos Duenhas Monreal<sup>4</sup>, Carla Giselly de Souza<sup>5</sup>, Renata Alves das Chagas<sup>5</sup> and Fernando Miranda de Vargas Junior<sup>5</sup>

<sup>1</sup>Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil. <sup>2</sup>Agência de Desenvolvimento Agrário e Extensão Rural, Dourados, Mato Grosso do Sul, Brazil. <sup>3</sup>Universidade Anhaguera, Campo Grande, Mato Grosso do Sul, Brazil. <sup>4</sup>Universidade Federal do Mato Grosso do Sul, Pioneiros, Mato Grosso do Sul, Brazil. <sup>5</sup>Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Rodovia Dourados-Itahum, km 12, 79804-970, Dourados, Mato Grosso do Sul, Brazil. <sup>\*</sup>Author for correspondence. E-mail: carlaxlsouza@yahoo.com.br

**ABSTRACT.** The objective was to evaluate fertility, cyclicality behavior, and sexual performance of Pantaneiro sheep throughout the year's photoperiods in Mato Grosso do Sul - Brazil. Two experiments were conducted. In experiment one, the ewes used aged between 2 and 4 years, distributed in three homogeneous groups of 54 animals and subjected to reproductive evaluation in three different predefined luminosity periods, consisting of autumn (11h 45 min.), spring (12h 30 min.), and summer (12h 55 min.). The characteristics studied were body weight (BW), body condition score (BCC), estrus distribution (EST), percentage of pregnant females per cycle (%P), plasma progesterone (PP), and fertility (F) in the three predefined seasons. The mating system used was controlled natural nocturnal and lasted for 45 days. Experiment two, evaluated the ram sexual activity. The males (10) were individually exposed to four ovariectomized ewes. The ewes cycled during evaluated seasons, and the highest fertility was observed in animals with BCC >2.75. The PP concentration differed among periods, and higher conception rates (F) were observed in autumn and spring. The rams (75%) had a medium of sexual performance when they performed more than two ejaculations in 20 min throughout the seasons. Pantaneiro sheep of both genders had a similar fertility index, with early and constant activity throughout the year, displaying no photoperiodism or seasonality.

Keywords: fertility; Sexual performance; reproductive seasonality.

Received on January 22, 2021. Accepted on April 5, 2021.

## Introduction

Patterns of genetic flow at domestic animals variation processes in domestic animals have long proven insightful for the domestication study, breed formation, population structure, and the selection consequences. Natural and artificial selection processes in domestic animals play crucial roles in maintaining the flexibility of animal genetic resources in facing local environment constraints (Baazaoui et al., 2020).

In developing countries, livestock genetic resource conservation greatly contributes to sustainable development and the security of livelihoods and food production, reducing hunger and poverty (Sassi-Zaidy, Maretto, Charfi-Cheikhrouha, Mohamed-Brahmi, & Cassandro, 2016). During a breed evolution, many characters, resulting from different selection pressures, become fixed. These come from climate changes, endemic parasites, diseases, and nutrition availability, as well as the influence of human groups. Historically, humans have selected animals based on the physical and/or productivity characteristics which best meet their cultural and economic needs. Thus, each breed is the result of both evolutionary processes, adapting to different environments, and the effect of human decisions and actions. Therefore, their DNA holds not only genetic information, but it may also be associated with historical and cultural information (Arandas et al., 2017).

Factors that may affect the reproductive activity of small ruminants include season, photoperiod, race, age, nutritional diseases, stress, management, environment, and male effects (Talafha & Ababneh, 2011). Sheep exhibit seasonal polyestrous and reproductive activity related to increasing or decreasing daylight.

Therefore, sheep are called short-day animals. One of the main factors responsible for this seasonality is the photoperiod, whose influence on the reproduction of females and males is interdependent with latitude,

breed, and nutritional status. Day-length changes reach animals through multiple neural pathways and, determine the seasonal secretion profiles of many hormones, including melatonin, prolactin, and gonadotropin (Molik, Misztal, Romanowicz, & Zieba, 2013).

Annual lamb production is higher in hair breed herds (Arandas et al., 2017) because them do not exhibit reproductive seasonality (annual polyestrous) this breeds are very resistant to parasites and adaptable to a tropical climate, cycling during the whole year or exhibiting a short anestrous period (Biehl et al., 2019). Additionally, they have a higher ovulation rate, and consequently, higher levels of prolificacy than do the wool sheep breeds (Santos et al., 2011).

The indigenous or autoctones sheep breeds have been identified for their rusticity and ability to adapt to regions of semi-arid, tropical, and subtropical climates in Brazil. The indigenous sheep genetic group from State of Mato Grosso do Sul (Pantaneiro sheep) presents allele combinations that approaches the local wool breeds from the south America and the hair breeds from the Brazilian Northeast. In this sense, studies on Pantaneiro sheep have highlighted that they experienced the natural selection process for many years, allowing the animals to adapt to the wet region. Thus, these sheep are considered locally adapted to State Mato Grosso do Sul conditions (Crispim, Seno, Egito, Vargas Junior, & Grisolia, 2014).

The lack of scientific information related to Pantaneiro sheep production is one obstacle to agricultural producers engagement to preserve this genetically adapted and endangered material. These sheep breed have good characteristics for industrial mating (maternal basis) with commercial sheep herds, helping farmers to produce meat and milk in a more rustic environment and with fewer infrastructure requirements. The objective of this study was to evaluate fertility, cyclicality behavior, and sexual performance of Pantaneiro sheep throughout the year seasonality.

# Material and methods

Two experiments were conducted using Pantaneiro sheep herd in Campo Grande-MS, in the midwestern region of Brazil belonging to the Ovine Technological Center (OTC) of School Farm from Anhanguera-Uniderp University (UNIDERP) at 20°33′52.6″S, 54°32′09″W, between September 2007 and August 2008.

The climate in the Campo Grande region is classified, according to Köppen, as a tropical type Aw, with average temperatures of 24.4°C during the hottest months (January and February) and 19.1°C during the coldest months (June and July). The average annual precipitation was 1.470 mm January, which is the wettest month (average 243 mm of rain and 81% relative humidity). August is the driest month (average of 40 mm of rain and 60% relative humidity) (Empresa Brasileira de Pesquisa Agropecuária [Embrapa], 2003). Rainfall during April–May was 59.9 mm and the historical average was 63.2 mm, during September–October it was 180.0 mm with a historical average of 161.1 mm and during February–March it was 89.0 mm with a historical average of 99.5 mm. In experiment 1, 162 females were collected from different regions of South Mato Grosso, aged between 2 (four teeth) and 4 years (eight teeth).

Breeding season occurred in three periods, based on the luminosity index: autumn (11h 45 min.), spring (12h 30 min.), and summer (12h 55 min.) (Soares & Batista, 2004). They were adapted to the nutritional management of the OTC before experiment begins. They continued with the same nutritional and sanitary management of the property. The selected animals were genetically similar to the naturalized Brazilian sheep and shared haplotypes of Creole breeds from southern Brazil and that of the hair breeds from Northeastern Brazil (Crispim et al., 2014).

Ewes were weighed and the body condition score was assessed on a scale of 1 (thinner) to 5 (fatter) (Soares & Batista, 2004). To experiment 1, 54 females were distributed into three balanced groups (mean age, four teeth; mean body condition score, 2; and mean live weight, 36 kg), and mated in three different periods of luminosity, consisting of autumn (11h 45 min.), spring (12h 30 min.), and summer (12h 55 min.). To breeding period, the sexual ratio was one male for 18 females. Three rams were andrological tested during the three breeding seasons.

The natural breeding lasted 45 d at nighttime and was controlled by using harnesses mating markers to identify female estrus. Forty-five days after the end of the reproductive season, pregnancy was diagnosticated by transabdominal ultrasound (Pie Medical Ultrasound) with a transectoral transducer at a frequency of 6.0 MHz. The diagnosis was considered positive by viewing the gestational sac and embryo, associated with an increase in uterine unfolding by liquids.

Blood samples from 30 ewes, 10 per group, were collected weekly in the morning from the beginning to the end of the reproductive period, using a vacuolized tube and packed in a polystyrene box in an ice bath. The plasma was immediately obtained by centrifugation at 3000 g rotations for 10 min. and stored at -20°C for further progesterone analysis. Progesterone hormonal profile evaluation using the radioimmunoassay technique with Cout-A-Count (DPC)<sup>®</sup>, Botucatu-SP hormonal kits. Ewes with progesterone levels above 0.5 ng mL-1 had ovulated. Hormonal levels below 1 ng mL<sup>-1</sup> for more than 10 d were characterized as females in anestrus (Brunet, Sebastian, Picazo, Cabellos, & Goddard, 1995).

The ewes were kept in *Brachiaria* sp. pasture, and in the night shift they were sheltered in the management center for breeding. There are 3 replicates per treatment. The comparison of the mean hormonal progesterone (P4) profile and mean live weight (BW) in the different seasons was performed using the Tukey's test at 5% probability. To test for differences among variables, consisting of mean body condition score (BCS), the number of estrus cycles, and conception rate in the different seasons, a chi-square test ( $\chi$ 2) was used.

In experiment two, ten Pantaneiro male sheep were used, six puberty males (approximately 1-year-old), and four adult males (2-4 years old, having four-eight permanent teeth). The animals remained under natural field conditions in paddocks of Massai grass (*Panicum maximum*- Massai), received water and mineral supplement *ad libitum*, and were supplemented during the dry season with ground corn and urea. Sanitary and preventive care, such as deworming and vaccinations, were performed to maintain good health.

The rams were subjected to a libido test in an area approximately 44 m2 during the morning period. The test consisted of exposing each male to four ovariectomized female for 20 min., which were induced into artificial estrus by synchronization with 2 mL estradiol benzoate (Estrogin®, Farmavet, São Paulo, SP), 48h before testing. The male libido evaluation was performed once a month, during the morning for 12 months, and classified according to sexual performance in performing jumps and ejaculations during trail.

The male sexual performance was evaluated according to the following criteria: 1) interest behavior in the females (Chemineau, Cognie, Guerin, Orgeur, & Vallet, 1991); 2) number of mount failing ejaculation and the total number of services, i.e., a number of matings lasting for 20 min., similar to that described by Price, Price, Erhard, Borgwardt, and Dally, (1992); 3) reaction time defined as the elapsed time from the exposure of male to female until the first service, similar to that described by Chemineau et al., (1991).

The rams were ranked according to Price et al., (1992), as sexually inactive (ISP) if only occurs jump without ejaculation. Low levels of sexual performance (LLSP) consisted of up to two ejaculations. Medium levels of sexual performance (MLSP) consisted of up to three ejaculations, and high levels of sexual performance (HLSP) were characterized by more than three ejaculations. The sequence of showing females on each month record differed throughout the experiment.

The statistical analysis was randomized blocks with subdivided plots, considering seasons as plots (spring, summer, autumn, and winter), and months of each season (September to August) as subplots, with repetitions consisting of animals (n = 10). The analysis of variance (ANOVA) was performed, with a significance level of 5%. If there was a significant difference demonstrated by the ANOVA, the means were compared using the Tukey test at 5%, according to Gomes (1985).

### **Results and discussion**

BW and BCS of the Pantaneiro ewes varied among seasons and within the same period. BCS values at the beginning and end of gestation were significantly higher (p <0.05) in autumn (11h 45 min.) and spring (12h 30 min.) than those recorded during summer (12h 55 min.). BW values at the end of pregnancy were higher (p <0.05) during spring (12h 30 min.) relative to those recorded in autumn (11h 45 min.) and summer (12h 55 min.). Among the factors that affected BW were lamb sex, ewe age, calving season and the interaction between these factors, and there was individual variation in BW (Table 1).

Inadequate nutrition of ewes, primarily during pregnancy, may limit lamb performance pre- and postweaning. Therefore, adequate nutrition for the ewe in pregnancy contributes to hypertrophy of primary muscle fibers that provide support and contribute to the hyperplasia of secondary fibers. Consequently, the high number of muscle fibers at birth promotes a high rate of weight gain in lambs (Sousa et al., 2018). In the present study, animals of the same genotype and sex were used, with homogeneous batches among groups, and it was observed that the type of food offered was decisive for the differences in BCS and BW among periods.

During summer, it was observed that the ewes from the middle to the end of the pregnancy lost weight. This was unexpected but can be explained by the final third of pregnancy occurring during the dry season,

#### Page 4 of 9

when pasture quality was not sufficient to meet the nutritional requirements of the animals, considering the permanence of the herd in the field condition. The ewes subjected to breeding in the spring, the period of greatest rainfall, lost less weight, and consequently, were heavier at birth (p < 0.05) compared with the pregnant ewes in the other two groups.

 Table 1. Averaged body condition score (BCS) and body weight (BW) followed by their respective standard errors at different stages of pregnancy according to the reproductive period of Pantaneiro ewes.

Daramatara	Reproductive Periods					
Parameters	Autumn	Spring	Summer	Mean		
BCS early pregnancy	$2.01 \pm 0.40^{Aa}$	$2.08\pm0.45^{\mathrm{Aa}}$	$1.75\pm0.53^{\rm Ab}$	$1.95 \pm 0.46$		
BCS mid-pregnancy	$2.15 \pm 0.36^{Ba}$	$2.36 \pm 0.50^{Ba}$	$2.18 \pm 0.56^{Ba}$	$2.23 \pm 0.47$		
BCS late pregnancy	$2.34 \pm 0.33^{Ca}$	$2.38 \pm 0.44^{Ba}$	$1.90 \pm 0.59^{Ab}$	$2.21 \pm 0.45$		
Mean	$2.16 \pm 0.36$	$2.27 \pm 0.46$	$1.94 \pm 0.56$			
BW early pregnancy(kg)	$33.7 \pm 5.3^{Ab}$	$37.7 \pm 5.7^{Aa}$	$37.1 \pm 8.3^{Aa}$	$36.2 \pm 6.4$		
BW mid-pregnancy (kg)	$35.8 \pm 5.5^{Bb}$	$39.9 \pm 5.9^{Ba}$	$37.0 \pm 8.5^{Aab}$	$37.6 \pm 6.6$		
BW late pregnancy (kg)	$37.9 \pm 5.3^{\text{Cb}}$	$42.4 \pm 6.0^{Ca}$	$37.4 \pm 10.1^{\text{Ab}}$	$39.3 \pm 7.1$		
Mean	$35.8 \pm 5.4$	$40.0 \pm 5.8$	$37.2 \pm 9.0$			

Means followed by different capital letters in the same column differed within the same station and means followed by lowercase different letters in the same line differed between reproductive periods by the Tukey test (p <0.05).

In such circumstances, a factor that may have contributed to the greater ewe weight in early and late pregnancy in the spring period was the greater rainfall.

The results indicated that ewes with BCS >2.75, than 2.0/2.5, and <2.0 had similar conception rates, being 100% (16/16), 80.7% (62/75), and 76.1% (54/71), respectively (p >0.05). However, that present study used indigenous ewes and controlled breeding, which may have been a factor that influenced good pregnancy results because of body condition. Some researchers when analyzing ewe fertility with different nutritional status have shown that low BCS reduces fertility (19 females), indicating that ruminant females must have a minimum of 2.5 BCS (scale 1-5) to obtain a minimum of 40% pregnancy. It has been reported that a BCS equal to or greater than 2.5 is related to an increase in luteinizing hormone (LH) pulsatility, promoting the return of cyclicality. BCS may also be related to the occurrence of estrus (Almeida et al., 2018).

The fertility of the Pantaneiro ewes was evident. Under suboptimal conditions, they achieved and sustained satisfactory pregnancy rates after mating. The ewe performance in terms of the number of estrus cycles accompanied by service in the different reproductive periods and the number of pregnant females in the different cycles are shown in Table 2.

		Reproductive Period					Total	
BCS*	I	Autumn		Spring	Summer		iotai	
	N	%	Ν	%	Ν	%	Ν	%
1.0	1	1.85	0	0.00	16	29.96	17	10.49
1.5	19	35.20	19	35.10	19	35.10	57	35.19
2.0	26	45.15	19	35.10	11	20.03	56	34.57
2.5	6	11.10	14	25.90	6	11.10	26	16.05
3.0	2	3.70	2	3.70	2	3.70	6	3.70
Total	54	100.00	54	100.00	54	100.00	162	100.00

**Table 2.** Number of estrus cycles, within the 45 d reproductive periods and the percentage of pregnancy according to the reproductive periods of Pantaneiro ewes.

BCS- body condition score; N- females who became pregnant.

The reproductive period did not showed significant effect (p > 0.05) on the number of estrus cycles over the 45 reproductive days. All animals cycled in all seasons, and the percentage of estrus time did not differ among reproductive periods (p > 0.05).

The ewes showed similar performance (p >0.05) per cycle at different reproductive periods. The results obtained were interesting because they showed that luminosity did not interfere with ewe's cyclicality and fertility at Midwest of Brazil where the study was conducted. Despite it was expected there would be a higher percentage of pregnant females in the first cycle with decreasing luminosity (autumn).

Talafha and Ababneh (2011) investigated the Awassi sheep breed, which is a autoctone breed to Jordan in Asia and the most important breed in the semi-arid regions of the Middle East countries observed no significant photoperiod effect. The Awassi sheep breeding season began in the fall and lasted until spring.

However, as the equator tropic is approached, reproductive seasonality is reduced, because the days are practically the same as the nights throughout the year. Thus, in subequatorial areas, such as the Brazilian Midwest, as long as there is nutritional support in sufficient quantity and quality, the ewe will manifest estrus throughout the year (Maia et al., 2017).

Although the ewes that participated in the autumn and summer reproductive periods expressed greater cyclical repeatability than the ewes in the spring season, the reproductive state did not differ (p > 0.05). This event proves a positive Pantaneiro ewe cyclicality in a period of increasing and transitional photoperiod, independent of body condition groups. Suggesting the absence of reproductive seasonality, likely with significant ovarian activity manifestation and does not support the hypothesis of seasonal anestrus for this type of ewe.

The results obtained support the information already described by Maia et al., (2017), who mentioned that cyclicality is also strongly influenced by breed origin, where Brazilian adapted goat/sheep exhibit reproductive activity throughout all the year. Even in areas close to the tropics, exotic breed sheep exhibit seasonality, as a consequence of the original local of breed effect. This was accomplished by the determination of female line origin through mitochondrial DNA sequencing, where it was verified that there was no significant difference between the group of females used in this experiment and the Brazilian naturalized breeds through mtDNA haplotype analysis. These animals presented haplotypes that brought them closer to the Brazilian wool breeds (H17), as well as to the local hair Brazilian breeds (H16) (Vargas Junior et al., 2014). This combination of genes naturally selected in the Pantanal biome for centuries, today through this research it can be phenotypically observed that they are animals with excellent reproductive performance.

Plasma progesterone concentrations (P4) of indigenous ewes differed among seasons (p <0.05), but the increase in P4 was observed in the three periods with breeding season advancement, despite intra-seasonal fluctuations (Figure 1). The ewes in the spring period exhibited lower P4 concentrations at the beginning of the reproductive period, being different (p <0.05) from that of autumn and summer. Pantaneiro ewe in the autumn and spring seasons, from 28 d after the reproductive period, had the highest average progesterone levels.



**Figure 1.** Progesterone (P4) hormone profile in the autumn, spring, and summer periods of Pantaneiro ewes. <sup>a/b</sup> Different letters for each harvest on the x-axis differed by the Tukey' s test (p <0.05).

Circulatory fluctuations and increased P4 concentrations have been described by Bartlewski, Beard, and Rawlings, (1999), being ostensibly lower during the first phase of the season compared to the P4 averages of the mid-season cycles in Western white-faced ewe. Despite the differences observed, the mean P4 was greater than 0.5 ng mL<sup>-1</sup> during the entire experimental period and in the three reproductive periods, and not less than 1 ng mL<sup>-1</sup> for a period of more than 10 d. These data indicate ovarian activity and absence of anestrus, confirming the observations made by Brunet et al., (1995).

Progressive increases in mean P4 concentrations characterized the endocrine response to conception, and according to Gray et al., (2006) are directly related to the early stage of pregnancy, originating from the corpus luteum. In the same study, they emphasized that P4 acts in the endometrium, regulating important genes for

uterine receptivity and growth of the fetus, and maintains plasma P4 elevation, which corroborates the results observed in this experiment. Table 3 shows the fertility indexes in the different reproductive periods studied, among which a similar conception rate was observed (p > 0.05).

Parameters –		Total		
	Autumn	Spring	Summer	- Iotai
Ewes number	54 (46)	52 (46)	53 (40)	159 (132)
Conception rate (%)	85.2a	88.5a	75.5a	83.0
Ewes in anestrus number	0	2	1	3
Ewes in anestrus (%)	0	3.8	1.9	1.9
Total	54	54	54	162

Table 3. Pantaneiro ewe's fertility in three different reproductive periods.

a/b Different letters in the same line differ by the chi-square test ( $\chi 2$ ) (p <0.05). Numbers in parentheses refer to ewes who became pregnant.

A study with natural breeding have documented 61% (Stellflug, 2002) fertility, which are lower than the values obtained in this experiment. In these studies, the authors used drugs with the ability to stimulate cyclic activity, an action not performed in this study, i.e., the ewes exhibited natural cyclic activity.

Concerning the males, the total matings and number of services were not significantly different (p < 0.05) among the seasons of the year for the total number of services. The Tukey test revealed a significant difference (p < 0.05) for the average of services between the months studied (Table 4).

**Table 4.** Average day luminosity and average of total ram, total of services, male interest score, and reactions time, followed by their respective standard errors, in indigenous Pantaneiro ram from spring to winter.

Season	Period	Day luminosity average (hours)	Total mating (NS)	Total of Services	Interest (NS)	Reaction time (seconds) (NS)
Spring	Sept-07	12:30	12.9 ± 4.38*	$2.2 \pm 0.41$ ABC	$3.3 \pm 0.33$	$63.0 \pm 21.03$
	Oct-07	12:55	$10.4 \pm 3.66$	$1.8 \pm 0.41$ <sup>BC</sup>	$3.2 \pm 0.32$	$135.0 \pm 60.50$
	Nov-07	13:20	$10.6 \pm 2.85$	$1.7 \pm 0.49^{\mathrm{BC}}$	$3.4 \pm 0.34$	$82.0 \pm 32.31$
Summer	Dec-07	13:30	$9.4 \pm 1.57$	$2.4 \pm 0.26$ ABC	$3.7 \pm 0.21$	$29.5 \pm 6.07$
	Jam-08	13:20	$14.6 \pm 4.59$	$2.6 \pm 0.37$ AB	$0.0 \pm 0.00$	$35.5 \pm 10.68$
	Feb-08	13:00	$12.8 \pm 3.13$	$2.2 \pm 0.46^{\text{ABC}}$	$4.0 \pm 0.00$	$66.5 \pm 17.16$
	Mar-08	12:55	$12.5 \pm 3.73$	$2.3 \pm 0.26^{\text{ABC}}$	$3.6 \pm 0.16$	$45.0 \pm 18.40$
Autumn	Apr-08	12:00	$15.0 \pm 6.21$	$2.6 \pm 0.42$ AB	$3.6 \pm 0.22$	39.0 ± 13.55
	May-08	11:40	$7.4 \pm 2.29$	$1.5 \pm 0.30^{\circ}$	$3.5 \pm 0.22$	$51.3 \pm 17.36$
Winter	Jun-08	11:00	$9.9 \pm 1.72$	$2.9 \pm 0.27$ <sup>A</sup>	$3.7 \pm 0.15$	$37.5 \pm 15.11$
	Jul-08	11:20	$19.4 \pm 3.62$	$2.9 \pm 0.58$ <sup>A</sup>	$3.7 \pm 0.15$	$24.8 \pm 2.20$
	Aug-08	11:50	$8.1 \pm 0.80$	$2.4 \pm 0.48$ ABC	$3.7 \pm 0.15$	$50.4 \pm 22.51$

The same letters in each column indicate no significant difference. ANOVA followed by Tukey's p test <0.05. \* Mean standard error. N = 10 ram. NS Not significant.

The shortest period of service observed was in late autumn (May), which is the period of greatest sexual activity for the ram that has reproductive seasonality (Santos et al., 2011). There was no correlation (p >0.05) between monthly service averages and luminosity average during the experimental period. Thus, it was possible to show that rams Pantaneiro do not have reproductive seasonality compared to wool sheep breeds with European heritage, which are seasonal polyestrous (Kuru, Sogukpinar, Makav, & Cetin, 2017).

The results indicated that the decrease in sexual activity (fewer services) did not occur during the longest photoperiod (spring and summer), showing only occasional and isolated declines. It was also observed that the males maintained the number of matings and services throughout the seasons. Additionally, as different male sheep ages were used, it was observed that young rams exhibited aggressiveness when approaching the ewes. This attitude can be explained by the lack of experience of the young male sheep. The number of matings exhibited an oscillating behavior, sometimes being low and sometimes high relative to adults, which maintained a more stable number of matings throughout the year.

In this sense, it can be suggested that the young ram, that lack experience relative to the older ones. However, that rams became less aggressive throughout the experiment. This behavior characterizes the gain in experience of the younger ram and is an important sign for the orientation in the selection of reproducers with satisfactory performance, even when young they show an early interest in females.

These results agree with the observations in the literature, wherein authors have indicated the need for prior sexual exposure of young rams to ewes in estrus before the breeding season, in order to improve their

sexual performance. Additionally, it is a pre-coital component of libido, wherein the rams 'tests' the receptivity of females to the sexual act (Pacheco & Quirino, 2010).

Regarding the sexual activity classification, seasonal effects were not evident (p > 0.05), but a specific classification pattern was observed for the number of services. Thus, the rams showed average levels of sexual performance most of the time (75%), corresponding to 9 of the 12 months studied among the seasons. Low levels of sexual performance were observed occasionally during brief time intervals (October, November, and May).

The findings reported here did not differ (p >0.05) among seasons. Therefore, Pantaneiro male sheep demonstrated satisfactory sexual performance throughout all year. However, the methods of measuring sexual performance levels differed greatly in the literature. Stellflug (2002) using controlled breeding in Polypay ewes (with prolonged seasonality) during spring, classified sexually active young males as having high levels of sexual performance with average services of 1.8 ( $1.8 \pm 0.3$ ; n = 5) and low levels of sexual performance with average services of 0.9 ( $0.9 \pm 0.2$ ; n = 5); this was lower than that found in the present study.

However, there is a need to standardize the evaluation methods to obtain comparison parameters between breeds. In Table 4, also the interest for the ewes can be observed. There was no influence (p > 0.05) of luminosity period on sexual interest.

The rams exhibited consistent behavior, with increased interest in ewes throughout the experimental period, indicating adaptation to the test, whereas the performance remained stable (inherent factors), corroborating the findings of Kridli and Said (1999). Regarding the reaction time, there was no significant difference (p > 0.05) for the seasons or luminosity, indicating that periods with greater luminosity did not influence the reaction time of the rams (Figure 2).



Figure 2. Effect of luminosity period at day (h) and sexual reaction time during the seasons for Pantaneiro rams.

A high negative correlation (r = -0.81) was observed between the reaction time and the total number of services, indicating that when the reaction time was shorter, the total number of services increased. This behavior is similar to that of sheep of European origin that have negative photoperiodism, i.e., they work best when there is a decrease in luminosity (Vargas Junior et al., 2014).

However, for the Pantaneiro sheep, the behavior in periods with greater light was similar to that of Santa Ines sheep, which also do not exhibit seasonality and may indicate a positive inherited characteristic because these animals are part of the composition of the Pantaneiro Ovine Genetics (Vargas Junior et al., 2014).

Reproductive seasonality is one of the obstacles to intensive sheep production (Kuru et al., 2017). Although sheep of other breeds and genetic groups present discreet photoperiodism, little is known about released sheep that have no reproductive seasonality. The existence of another genetic group of sheep with no seasonality opens new horizons in sheep farming because reproductive management is flexible, allowing breeding stations adapted to different situations within a sheep and meat production farm (Wanapat, Cherdthong, Phesatcha, & Kang, 2015).

### Conclusion

The rams and ewes Pantaneiro have fertility index, with early and constant activity throughout the year, with no photoperiodism or seasonality.

# Acknowledgements

We thank the Manoel de Barros Foundation for funding the research and the Centro Tecnológico de Ovinocultura - CTO from UNIDERP- Anhanguera, Campo Grande, MS, for allowing the use of its facilities to conduct the experiments.

# References

- Almeida, S. F. C. M., Souza-Fabjan, J. M. G., Balaro, M. F. A., Bragança, G. M., Pinto, P. H. N., Almeida, J. G.,
  ... Brandão, F. Z. (2018). Use of two doses of cloprostenol in different intervals for estrus synchronization in hair sheep under tropical conditions. *Tropical Animal Health and Production*, *50*(2), 427-432.
  DOI: https://doi.org/10.1007/s11250-017-1454-x
- Arandas, J. K. G., Alves, Â. G. C., Facó, O., Belchior, E. B., Shiotsuki, L., Arruda, P. M. B. L., & Ribeiro, M. N. (2017). Do traditional sheep breeders perform conscious selection? An example from a participatory breeding program of Morada Nova sheep. *Tropical Animal Health and Production*, 49(7), 1479-1487, 30. DOI: https://doi.org/10.1007/s11250-017-1350-4
- Baazaoui, I., McEwan, J., Anderson, R., Brauning, R., McCulloch, A., Van Stijn, T., ... Romdhani, S. (2020).
   GBS data identify pigmentation-specific genes of potential role in skin-photosensitization in two tunisian sheep breeds. *Animals*, *10*(1), 1-10. DOI: https://doi.org/10.3390/ani10010005
- Bartlewski, P. M., Beard, A. P., & Rawlings, N. C. (1999). Ovarian function in ewes at the onset of the breeding season. *Animal Reproduction Science*, 57(1-2), 67-88. DOI: https://doi.org/10.1016/S0378-4320(99)00060-3
- Biehl, M. V., Ferraz Junior, M. V. C., Barroso, J. P. R., Susin, I., Ferreira, E. M., Polizel, D. M., & Vaz Pires, A. (2019). The reused progesterone device has the same effect on short or long estrus synchronization protocols in tropical sheep. *Tropical Animal Health and Production*, *51*(6), 1545-1549. DOI: https://doi.org/10.1007/s11250-019-01841-1
- Chemineau, P., Cognie, Y., Guerin, Y., Orgeur, P., & Vallet, J. C. (1991). Collection and preservation of spermatozoa. In P. Chemineau, *Training Manual on Artificial Insemination in Sheep and Goats* (p. 115-130). Rome, IT: Food & Agriculture Organization of the United Nations.
- Crispim, B. A., Seno, L. O., Egito, A. A., Vargas Junior, F. M., & Grisolia, A. B. (2014). Application of microsatellite markers for breeding and genetic conservation of herds of *Pantaneiro Sheep.*, 17(6), 317-321. DOI: https://doi.org/10.1016/j.ejbt.2014.09.007
- Empresa Brasileira de Pesquisa Agropecuária [Embrapa]. (2003). *Bancos de dados climáticos do Brasil*. Retrieved from https://www.embrapa.br/busca-de-publicacoes/-/publicacoa/939638/base-de-dados-climaticos
- Gomes, F. P. (1985). Curso de estatística experimental. Piracicaba, SP: Livraria Nobel.
- Brunet, A. G., Sebastian, A. L., Picazo, R. A., Cabellos, B., & Goddard, S. (1995). Reproductive response and LH secretion in ewes treated with melatonin implants and induced to ovulate with the ram effect. *Animal Reproduction Science*, *39*(1), 23-34. DOI: https://doi.org/10.1016/0378-4320(94)01368-V
- Gray, C. A., Abbey, C.A., Beremand, P. D., Choi, Y., Farmer, J. L., Adelson, D. L.,... Spencer, T. E. (2006). Identification of Endometrial genes regulated by early pregnancy, progesterone, and interferon tau in the ovine uterus. *Biology of Reproduction*, *74*(2), 383-394. DOI: https://doi.org/10.1095/biolreprod.105.046656
- Kridli, R. T., & Said, S. I. (1999). Libido testing and the effect of exposing sexually naive Awassi rams to estrous ewes on sexual performance. *Small Ruminant Research*, *32*(2), 149-152.
  DOI: https://doi.org/10.1016/S0921-4488(98)00168-0
- Kuru, M., Sogukpinar, O., Makav, M., & Cetin, N. (2017). Effect of barium selenate injections on fertility of Pirlak ewes subjected to estrus synchronization during non-breeding season. *Medycyna Weterynaryjna*, 73(8), 479-482. DOI: https://doi.org/10.21521/mw.5758.
- Maia, A. L. R. S., Oliveira, M. E. F., Souza-Fabjan, J. M. G., Balaro, M. F. A., Brandão, F. Z., & Fonseca, J. F. (2017). Distúrbios reprodutivos em cabras leiteiras e impactos potenciais nos sistemas de produção. *Revista Acadêmica Ciência Animal, 15*(Suppl 2), 77-89. DOI: https://doi.org/10.7213/academica.15.S02.2017.A08
- Molik, E., Misztal, T., Romanowicz, K., & Zieba, D. (2013). Short-day and melatonin effects on milking parameters, prolactin profiles and growth-hormone secretion in lactating sheep. *Small Ruminant Research, 109*(2-3),182-187. DOI: https://doi.org/10.1016/j.smallrumres.2012.10.006

- Pacheco, A., & Quirino, C.R. (2001). Sexual behavior of ram. *Revista Brasileira de Reprodução Animal, 34*(2), 87-97.
- Price, E. O., Price, E. O., Erhard, H., Borgwardt, R., & Dally, M. R. (1992). Measures of libido and their relation to serving capacity in the ram. *Journal of Animal Science*, *70*(11), 3376-3380. DOI: https://doi.org/10.2527/1992.70113376x
- Santos, G. M. G., Silva-Santos, K. C., Melo-Sterza, F. A., Mizubuti, I. Y., Moreira, F. B., & Seneda, M. M. (2011). Desempenho reprodutivo de ovelhas mestiças lanadas e deslanadas submetidas a protocolo hormonal a base de progestágeno e eCG, durante a contraestação reprodutiva. *Semina: Ciências Agrárias,* 32(2), 723-732. DOI: https://doi.org/10.5433/1679-0359.2011v32n2p723
- Sassi-Zaidy, Y. B., Maretto, F., Charfi-Cheikhrouha, F., Mohamed-Brahmi, A., & Cassandro, M. (2016). Contribution of microsatellites markers in the clarification of the origin, genetic risk factors, and implications for conservation of Tunisian native sheep breeds. *Genetics and Molecular Research*, *15*(1), 1-11. DOI: https://doi.org/10.4238/gmr.15017059
- Soares, R. V., & Batista, A. C. (2004). *Meteorologia e climatologia florestal*. Curitiba, PR: Departamento de Ciências Florestais da UFPR.
- Sousa, S. V., Araújo, M. J., Silva, T. P. D., Marques, C. A. T., Costa Torreão, J. N., Bezerra, L. R., ... Gottardi, F. P. (2018). Dietary supplementation for Santa Inês hair ewes on pasture at pre- and postpartum periods: dry matter intake, digestibility, milk production, and mineral metabolism. *Tropical Animal Health and Production*, *50*(8), 1903-1912. DOI: https://doi.org/10.1007/s11250-018-1643-2
- Stellflug, J. N. (2002). Influence of classification levels of ram sexual activity on spring breeding ewes. *Animal Reproduction Science*, *70*(3-4), 203-214. DOI: https://doi.org/10.1016/S0378-4320(02)00008-8
- Talafha, A. Q., & Ababneh, M. M. (2011). Awassi sheep reproduction and milk production: Review. *Tropical Animal Health and Production*, *43*(7), 1319-1326. DOI: https://doi.org/10.1007/s11250-011-9858-5
- Vargas Junior, F. M., Martins, C. F., Santos Pinto, G., Ferreira, M. B., Almeida Ricardo, H., Leão, A. G., ... Teixeira, A. (2014). The effect of sex and genotype on growth performance, feed efficiency, and carcass traits of local sheep group Pantaneiro and Texel or Santa Inês crossbred finished on feedlot. *Tropical Animal Health and Production*, 46(5), 869-875. DOI: https://doi.org/10.1007/s11250-014-0579-4
- Wanapat, M., Cherdthong, A., Phesatcha, K., & Kang, S. (2015). Dietary sources and their effects on animal production and environmental sustainability. *Animal Nutrition*, 1(3), 96-103. DOI: https://doi.org/10.1016/j.aninu.2015.07.004