Tropical Animal Health and Production
https://doi.org/10.1007/s11250-018-1696-2

REGULAR ARTICLES

Reproductive seasonality in Saanen goats kept under tropical conditions

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Received: 6 May 2018 / Accepted: 15 August 2018

Abstract
This study aimed to verify the reproductive seasonality in Saanen goats from distinct parity orders (nulliparous, primiparous, and pluriparous) throughout an entire year in the Southeast Region of Brazil. In addition, it was also verified the influence of environmental indexes, metabolic profile, body weight, and social interactions on the reproductive seasonality of these animals. Forty Saanen goats kept under intensive system at latitude 22° 52′ 30″ s and tropical photoperiod (11–13 h of light per day) were used. Every 15 days, blood from 24 animals (8 animals per group) was collected for assessment of serum progesterone levels. Monthly, environmental data (rainfall index, maximum and minimum temperature, and time of natural daylight) and blood were collected to evaluate the metabolic profile. Weighting and body condition score measurement were also carried out every 2 months. There was no difference in body weight and body condition score in primiparous and pluriparous goats. Nulliparous goats showed natural breeding season in the winter season while the primiparous and pluriparous goats remained cyclical in autumn and winter season. Regardless of the parity order, all goats showed a seasonal anestrus in the spring season. From December, social interactions were able to reestablish, in distinct degrees, the cyclical state in the goats. Likewise, environmental and weight indexes had low and moderate influence, respectively, on the reproductive seasonality in the Saanen goats kept under tropical conditions.

Keywords Body weight · Metabolic profile · Sexual behavior · Social interactions · Progesterone

Introduction

The first humans were nomadic and adapted to the seasonal availability of animal products (Balasse et al. 2003). Throughout the process of domestication, the seasonality of many livestock products was lost. Nevertheless, products as milk and meat provided by sheep and goat farming remained seasonal in many regions of the world (Chemineau et al. 2007). Native goats from tropical areas have shown low or even absence of reproductive seasonality (Ndeke et al. 2015), even though most tropical breeds do not show a genetic improvement for milk production as demonstrated by European dairy breeds. Therefore, attempts have been made to incorporate specialized breeds into the tropical climate and to make the dairy goat farming profitable.

Exogenous hormones (progestins, eCGs, prostaglandins, melatonin, etc.) have often been used to avoid seasonality and to induce reproductive activity during the anestrus time (Fatet et al. 2011). However, in recent years, many countries in the European Union have adopted restrictions on the use of synthetic hormones in livestock farming due to consumer demand for natural, green, clean methods (Rana and Paul 2017). Therefore, the knowledge of the annual reproductive rhythm of European goat breeds raised under tropical conditions is a major issue, in order to reduce the use of hormones.

It is known that when the daylight shows low variation, as seen in tropical and subtropical latitudes, other events are responsible to regulate the reproductive rhythm (Chemineau et al. 2004), such as nutrition (Gallego-Calvo et al. 2014), sociosexual interactions (Delgadillo et al. 2015), and climatic aspects (Silva et al. 1998; Morales et al. 2016). The association of all of them seems to be essential components in the brain plasticity.

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Published online: 29 September 2018

In this sense, it was aimed to study the reproductive seasonality in Saanen goats from distinct parity orders throughout a year in the Southeast region of Brazil. Moreover, the influence of some environmental indexes and body weight on the goats' reproductive seasonality was investigated.

Materials and methods

This study and procedures were approved by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (protocol no. 807).

Location, experimental animals, and social interactions

This study was carried in a dairy goat farm located in the Rio de Janeiro State, Brazil (latitude 22° 52′ 30” S; semi-humid tropical–Aw) from March 2015 to March 2016. A total of 40 Saanen goats were used: 12 pubertal nulliparous (0.8 ± 0.2 years old), 14 primiparous (2.9 ± 0.3 years old), and 14 pluriparous goats (5.4 ± 1.1 years old). All animals were kept under intensive system and fed daily with 4 kg/per goat of cropped grass (*Pennisetum purpureum*) and 3 kg/per goat of brewer waste in the wet form. Besides, it was given a concentrate with 22–24% and 18% crude protein for primiparous/pluriparous and nulliparous goats, respectively, totaling 0.8 to 1.3 kg/per dairy goat/day and 0.4 to 0.6 kg/per nulliparous goat/day. Water and mineral salt were available ad libitum.

Regarding social interactions, primiparous and pluriparous goats were kept in three different pens according to their milk yield. Nulliparous goats were kept together throughout the study. Breeding management was performed in two periods: (1) between March 18 and May 30, when experimental does were subjected to non-experimental ones, under natural estrus or synchronized with prostaglandins, and to the bucks (visual, olfactory and audible) when the same were entered from their barn (100 m away) to mating non-experimental does in estrus; (2) between days 11–12 and 18–19 December, when experimental nulliparous goats were exposed to other non-experimental goats kept on the same pen that were induced and synchronized to estrus. At this time, these non-experimental goats in estrus were taken to the buck's pens for mating. Thus, bucks did not have access to the barn or solarium where female flock was kept. It is important to highlight that no experimental animal was mated during all periods described previously.

Meteorological indexes

Daily, the rainfall index, and the maximum and minimum temperature time of natural daylight were collected from the web (http://www.climatempo.com.br/; https: //www.timeanddate.com/). The monthly average of these climatic indexes was used to characterize the local environmental conditions (Fig. 1).

Weight gain and body condition score

Bimonthly, all animals were weighed and the body condition score (BCS) was evaluated according to Detweiler et al. (2008).

Biochemical, hormonal, cyclic status, and sexual behavior evaluation

Fortnightly, the blood of 24 goats (8 animals per parity order; $n = 24$) was collected by jugular venipuncture in vacuum tube without anticoagulant for progesterone evaluation. In addition, blood samples were collected monthly from all goats ($n = 40$) for the evaluation of biochemical parameters. After blood collection, the samples were centrifuged (2600x*g*) and serum or plasma stored at −20 °C. Biochemistry measurements were carried out spectrophotometrically by the use of commercial kits (Labtest, Labtest Diagnostica AS, Minas Gerais, Brazil) in an automatic biochemical analyzer. The biochemical values were interpreted according to values proposed by Mundim et al. (2007). Serum P4 levels were evaluated by solid phase radioimmunoassay technique by using commercial kits (ImmuChem, MP Biomedicals, Santa Ana, CA, USA). The sensitivity and intra-assay coefficient were 0.05 ng/mL and 12%, respectively. All data were within the maximum and minimum point of the curve.

Goats' cycle was interpreted according to Minton et al. (1991) in which two consecutive evaluations (14 days apart) with serum progesterone above 1.0 ng/mL indicated the presence of a functional CL and the active cyclic state of the animal. The detection of the sexual behavior (bleating, flagging of the tail, reddened vulva, vaginal discharge, and accepted mounting by the bucks) was done daily by the local employee (previously trained) at the feeding time in the afternoon and data were organized and classified as the presence of animals in estrus or not (yes/no) per month of study.

Statistical analysis

Data were analyzed using the statistical program for statistical analyzes (SAEG 9.0, Universidade Federal de Viçosa, Minas Gerais, Brazil). Lilliefors test was used to verify the normality of variables. Parametric variables were analyzed using a mixed model procedure for repeated measures and the Tukey's test was used to compare means. Non-parametric variables were submitted to the Kruskal–Wallis test and Dunn's mean comparison. Categorical data (cyclic status) were assessed by the Fisher's exact test. The Pearson correlation coefficient was used to compare P4 levels with environmental,

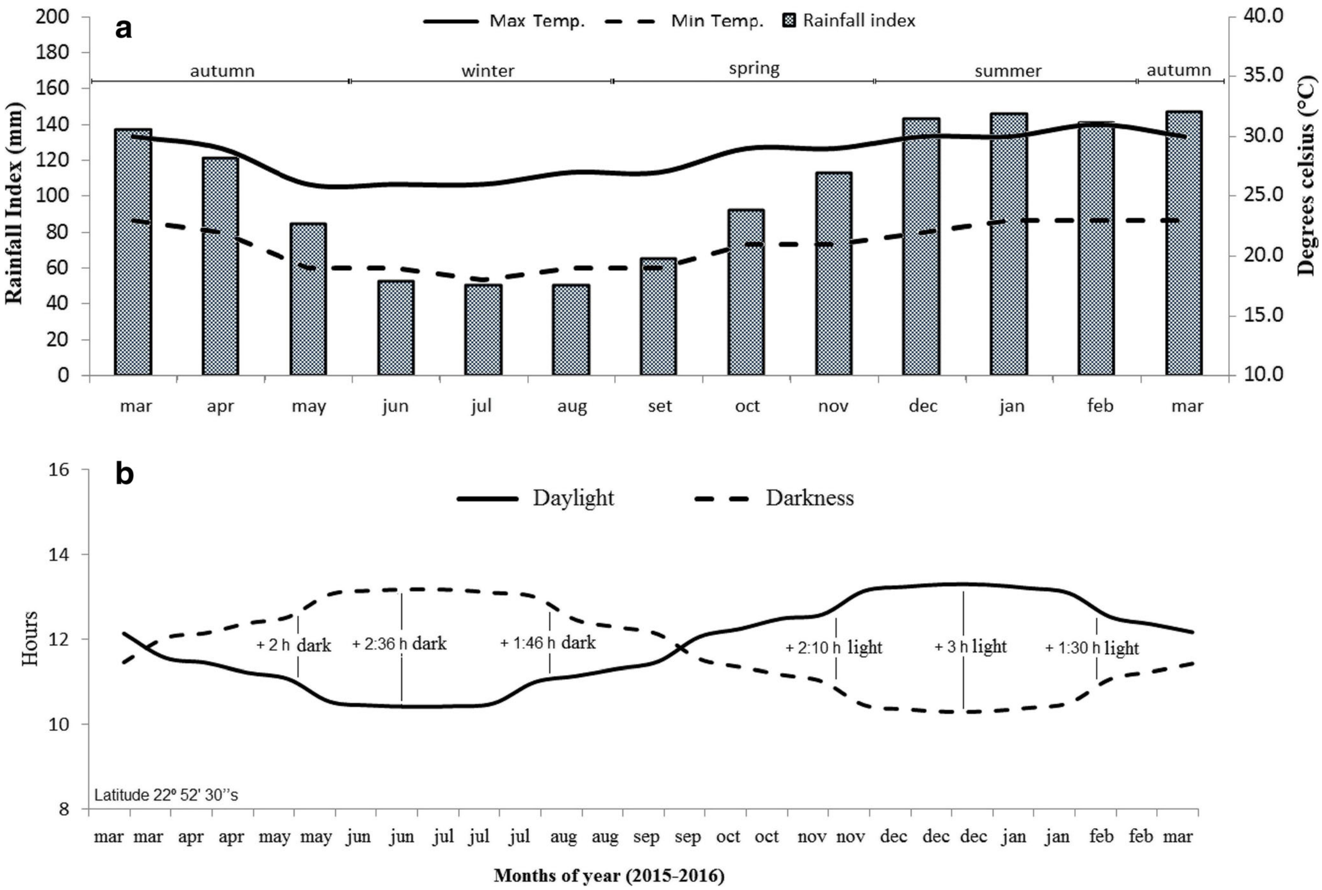


Fig. 1 Meteorological indexes throughout an entire year (March 2015–March 2016). A: rainfall index (mm) and maximum and minimum temperatures (°C). B: length of daylight and darkness (hours)

metabolic, and weight data by parity order. For all tests, $p < 0.05$ was considered significant.

Results

Weight gain and body condition score

The weight gain and BCS by parity order throughout the year are presented in Fig. 2. The annual average body weight was affected by the parity order (79.4 ± 10.1 vs 73.4 ± 10.5 vs 45.2 ± 6.7 kg, pluriparous, primiparous, and nulliparous, respectively, $p < 0.05$), as well as the BCS (3.5 ± 0.3 = 3.4 ± 0.3 vs 3.2 ± 0.2 BCS; $p < 0.05$). There was no difference in the body weight between the pluriparous and primiparous goats, but both were greater than nulliparous goats at all evaluation times.

Biochemical, hormonal, cyclic status, and sexual behavior evaluation

It was verified the effect of month on serum P4 levels in all parity order (Fig. 3). Pluriparous and primiparous goats showed greater serum P4 ($p < 0.05$) than nulliparous goats in the mid-March and May. In April, nulliparous goats exhibited a gradual increase of P4 with a peak between June and July and entry into the seasonal anestrus from the late July until the mid-December, when they return to cyclical activity. Primiparous and pluriparous goats showed a rapid increase on P4 levels from the mid-March, maintaining stable levels until the mid-August, when the seasonal anestrus was started and it was lasted until mid-December, when there was an elevation in P4 levels.

There was a month effect on the cyclicity pattern of all parity orders evaluated (Fig. 4). Pluriparous and primiparous goats showed a similar pattern of cyclicity with the breeding season from March to August, seasonal anestrous from September to November, and gradual return to the cyclical status from December. Nulliparous goats showed a greater proportion of cyclic animals in June and July, seasonal anestrous from August to November, and the total return (100% of the animals) of the reproductive activity in December with maintenance of this activity, in many animals, until March. Regarding the sexual behavior, goats showed all estrus signs composed by bleating, flagging of the tail, reddened vulva, vaginal discharge, and, mainly, accepted mounting by the bucks from March to June and in December.

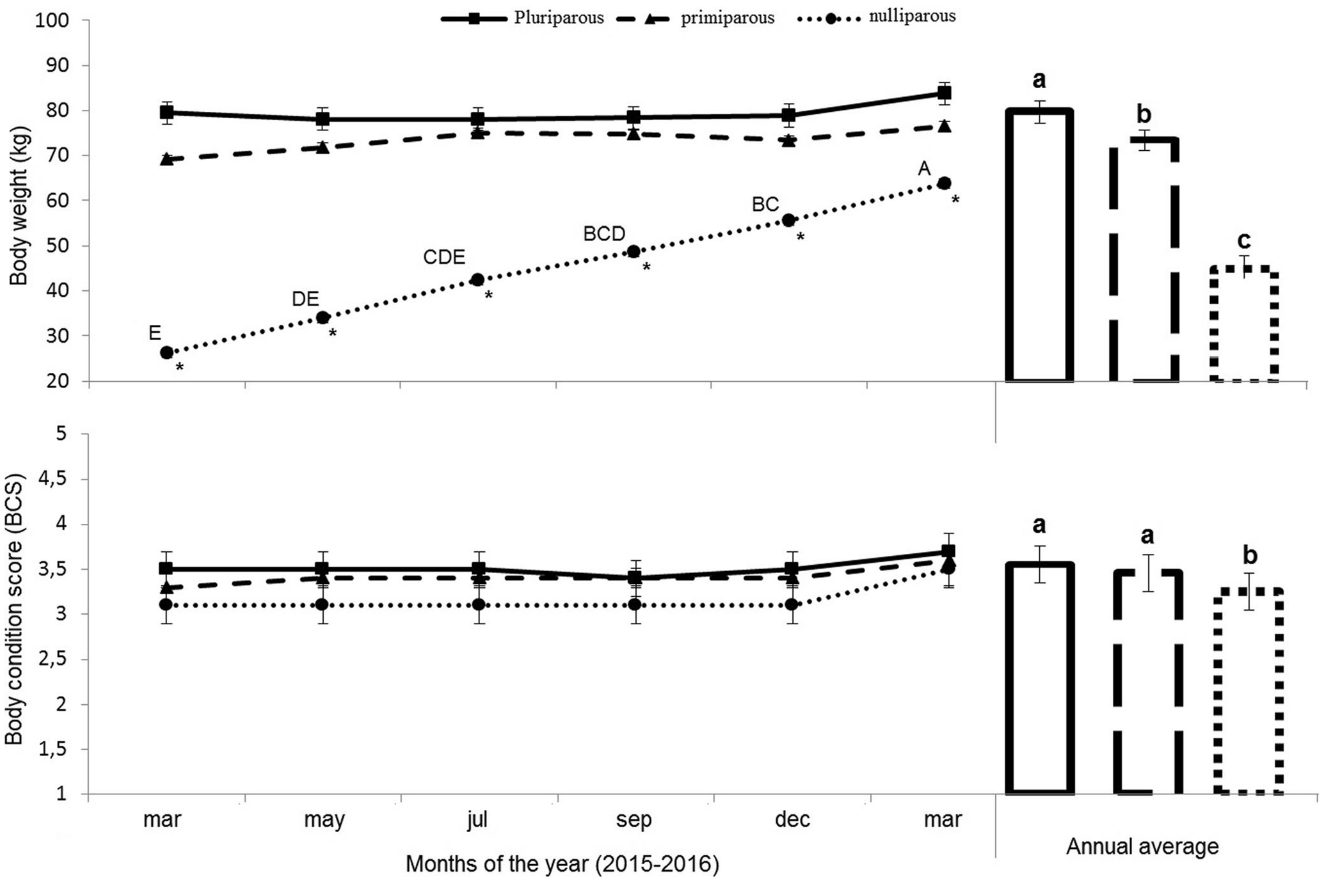


Fig. 2 Body weight and body condition score (BCS) of pluriparous, primiparous, and nulliparous Saanen goats ($n = 40$) throughout the study. A, B, C, D, E Distinct in time, within the same parity order, indicate significant difference by Tukey's test ($p < 0.05$). a, b, c, or * Distinct among parity orders, at the same time, indicate significant difference by Tukey's test ($p < 0.05$)

It was found that some biochemical indexes showed a season and parity orders effect throughout the study (Fig. 5). Besides, it is an important highlight that most of the biochemical indexes remained stable over the four seasons, as well as with the exception of the serum calcium, nulliparous goats showed a distinct pattern of metabolic profile when compared to primiparous and pluriparous goats. Although, the overall average of all the Saanen goats remains within the reference values.

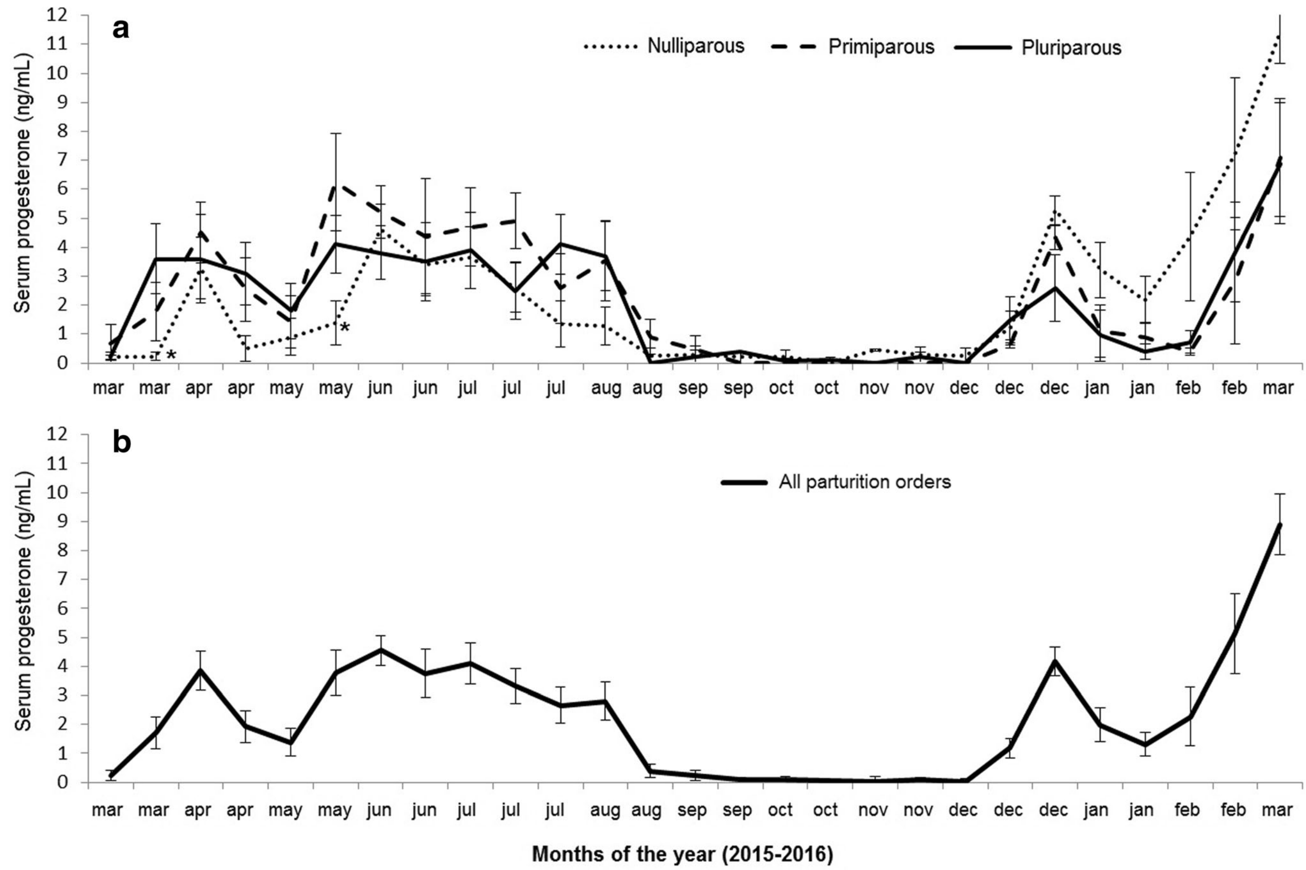
Correlations of serum progesterone with the body weight and meteorological indices

Correlations among serum progesterone with weight, BCS, and meteorological indexes in Saanen goats by distinct parity orders is presented in the Table 1. The body weight and BCS had positive correlations with P4 levels in nulliparous and primiparous goats. Interestingly, data of maximum and minimum temperature and rainfall index had a weak significant correlation with P4, but daylight and darkness had moderate correlations with P4 in primiparous and pluriparous goats throughout the study.

Discussion

In this study, a pattern of reproductive cyclicity was observed from autumn to winter in primiparous and pluriparous goats against the timing of cyclic nulliparous goats only in winter. Seasonal anestrus was well characterized in spring. Interestingly, in the early summer, under the influence of close social interactions, all nulliparous goats and some primiparous and pluriparous goats expressed sexual behavior. Equally, most of nulliparous goats and few primiparous and pluriparous goats demonstrated a persistence of cyclicity until early autumn. In this sense, it is well known that the expression of a given trait (phenotype) in an animal depends on the combined influence of intrinsic factors marked by genetics (genotype) and extrinsic factors, mainly environmental factors. In goats, the main environmental factors that influence reproduction are temperature, humidity, amount and distribution of rains, solar radiation, photoperiod, nutrition, management of the production system, and social interactions among individuals within the same population (Chemineau et al. 2004; Morales et al. 2016).

With regard to environmental factors, the pluviometric index and the maximum and minimum temperature had

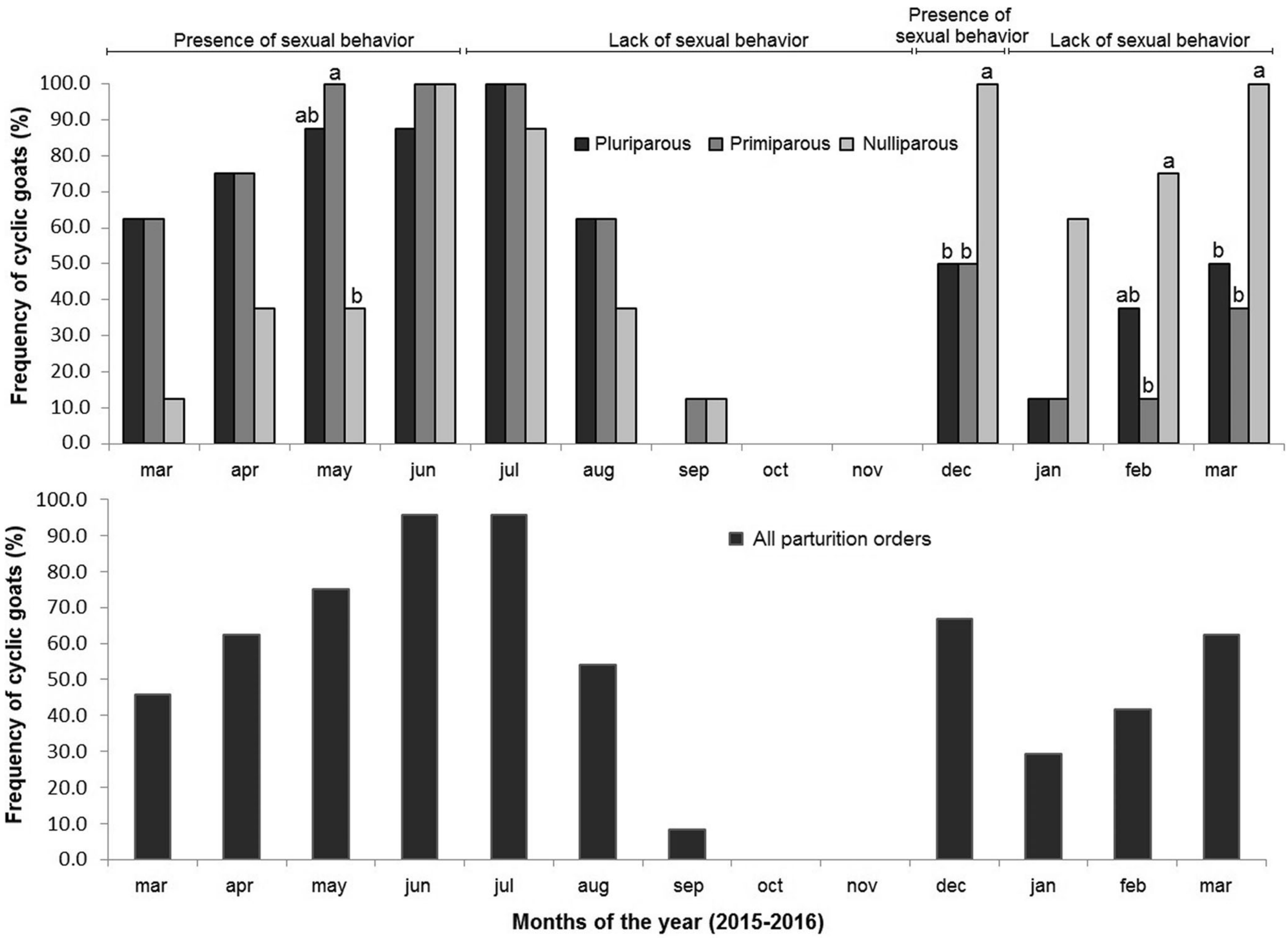


Months	mar	mar	apr	apr	may	may	jun	jun	jul	jul	jul	aug	aug	sep	sep	oct	oct	nov	nov	dec	dec	dec	jan	jan	feb	feb	mar
Nulliparous	d	d	b	d	cd	cd	ab	bc	bc	bc	cd	cd	d	d	d	d	d	d	d	d	cd	b	b	bc	b	ab	a
Primiparous	bc	ab	a	ab	bc	a	a	a	a	a	ab	ab	bc	c	c	c	c	c	c	c	bc	a	bc	bc	c	ab	a
Pluriparous	c	a	a	a	ab	a	a	ab	a	ab	a	a	c	c	c	c	c	c	c	c	ab	ab	bc	c	bc	a	a
All	c	bc	ab	bc	bc	ab	ab	ab	ab	ab	b	b	c	c	c	c	c	c	c	c	bc	ab	bc	bc	ab	ab	a

Fig. 3 Serum progesterone (P4) levels throughout the study (2015–2016) in Saanen goats ($n = 24$) by (A) parity order (pluriparous, primiparous and nulliparous) and (B) all together. * Indicates difference ($p < 0.05$) in P4 levels of nulliparous goats compared to pluriparous and primiparous goats. a, b, c, d Distinct among months indicates significant difference by the Tukey's test ($p < 0.05$)

weak correlations and little influence on the cyclic state of the goats. Morales et al. (2016) also reported a negative correlation of such indexes on the cyclicity of Mexican Crioula goats (22° N). Although Lopes Junior et al. (2001) and Silva et al. (1998) reported a positive correlation between rainy season and goat's cyclicity. In these studies, the quality of annual nutritional management was affected by the climatic condition and thus, the nourishment nutrition could have affected the cyclical state of those animals.

The local photoperiod, which ranged from 11 to 13 h of light per day, moderately influenced the seasonal goats' response. Interestingly, the number of hours of light and darkness correlated positively and negatively with P4 levels in all parity orders with the greatest influence on pluriparous and primiparous goats ($r = 0.42$; $p < 0.01$) when compared to nulliparous goats ($r = 0.12$; $p < 0.01$). This finding, interestingly corroborates with Delgadillo et al. (2011), regarding the refractoriness of long and short days that would be the regulating factor in the transition between the beginning and ending of the breeding season in goats under subtropical conditions and not the direct sensitivity to the increase or decrease of the local light stimulus as used to thought. Moreover, the breeding season of all parity orders was concentrated in the winter, a time of least luminous intensity (2:36 h more darkness) and where the length of the daylight begins to increase at the winter ending. Thus, the refractoriness of the light fall may have been responsible for the transition to anestrus. Also an important highlight is the difference in the annual cyclical status among nulliparous goats and the others parity orders. The fact that pluriparous and primiparous goats had a longer cyclical time may be related to a longer life and local adaptation to the environmental conditions (Chemineau et al. 2004), which could reduce the influence on their endogenous rhythm (Hazlerigg et al. 2004).



Months	mar	apr	may	jun	jul	aug	sep	oct	nov	dec	jan	feb	mar
Nulliparous	ab	a	a	a	a	ab	c	c	c	b	bc	bc	b
Primiparous	ab	a	a	a	a	ab	bc	c	c	b	bc	bc	bc
Pluriparous	bc	bc	bc	a	a	bc	bc	c	c	a	ab	ab	a
All	cd	bc	ab	a	a	bcd	e	e	e	bc	d	cd	bc

Fig. 4 Frequency of cyclicity (%) in association with the presence or absence of sexual behavior throughout the year (2015–2016) in pluriparous, primiparous, and nulliparous Saanen goats ($n = 40$). a, b Distinct among columns, at the same month, indicates significant difference by the Fisher's exact test ($p < 0.05$). a, b, c, d Distinct among months, at the same category, indicates significant difference by the Fisher's exact test ($p < 0.05$)

Nevertheless, such rhythm could still influence young animals as the nulliparous goats.

The body weight and BCS did not show annual variations in the pluriparous and primiparous goats, which can characterize a nutritional stabilization on them. As demonstrated in previous studies (Estrada-Cortes et al. 2009; Morales et al. 2016), body weight and BCS had mild to moderate positive correlations in primiparous and nulliparous goats, respectively, with serum P4 levels. These results highlight the importance of the good nutritional management and its projection on reproduction.

As almost all biochemical indices remained stable or with few variations, within the reference values, throughout the seasons, the main finding was a metabolic profile of nulliparous goats distinct to the others parity orders. This result may be justified by the physiological moment of primiparous and pluriparous goats, which were lactating throughout all experimental period. Therefore, it is expected that their metabolism to be distinct from young growing nulliparous goats as previously described (Antunović et al. 2017).

Social interactions such as female and male effect played an important role in this study. Initially, the continual visit of the buck to the goats' barn from mid-March to late May possibly contributed that about 90% and 100% of the primiparous and pluriparous goats, respectively, were cyclical by May and accompanied by sexual behavior until June. Such estrus

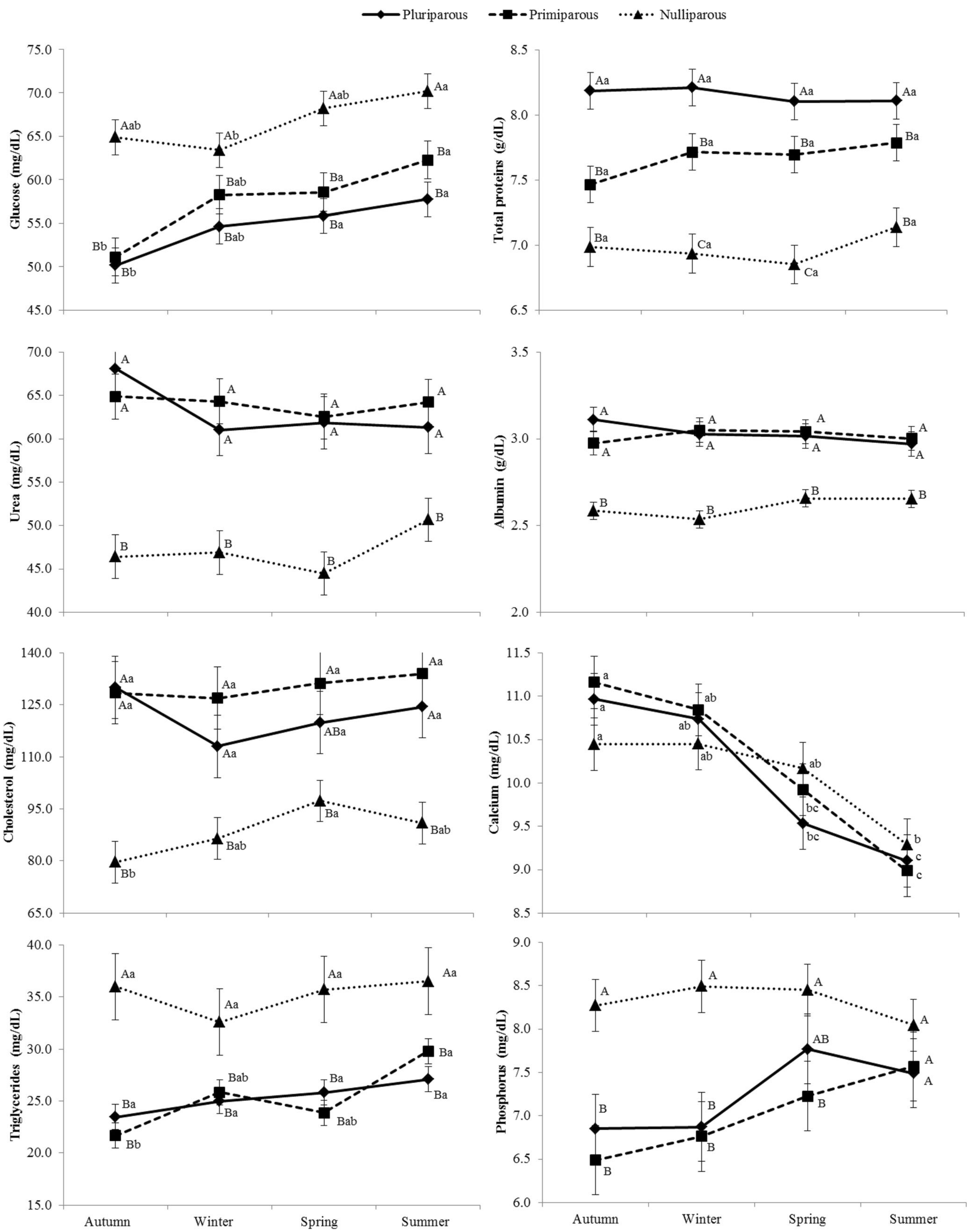


Fig. 5 Serum biochemistry (metabolic profile) of pluriparous, primiparous, and nulliparous Saanen goats ($n = 40$) by season (autumn, winter, spring, and summer). a, b, c Distinct in the same line, indicates significant difference by the Tukey's test ($p < 0.05$). A, B, C Distinct among lines, indicates significant difference by the Tukey's test ($p < 0.05$)

Table 1 Correlations among serum progesterone with weight, BCS, and meteorological indexes in Saanen goats ($n = 24$) apart by parity orders

Indexes	Serum progesterone (P4)			Total
	Nulliparous	Primiparous	Pluriparous	
Body weight	0.560	0.337	ns	0.256
BCS	0.536	0.281	ns	ns
Max. temp.	0.153	− 0.281	− 0.158	− 0.07
Min. temp.	0.186	− 0.210	ns	ns
Daylight	0.117	0.443	0.403	− 0.293
Darkness	− 0.125	− 0.446	− 0.406	− 0.298
Rainfall	0.180	− 0.199	ns	ns

ns not significant

behavior lasted for a month (until June) after the buck left the barn and ovulations without estrus manifestation (confirmed by P4 assessment) still happening by 3 months until the onset of anestrus in early September. Moreover, although the breeding season of the primiparous and pluriparous goats occurred in the entire period of autumn and winter, it was only noticeable until June which their sexual behavior ceased. In this context, Delgadillo et al. (2015) demonstrated that continuous social stimulation in does (26^{o} N) by active bucks (pretreated with photoperiod or melatonin implants) was able to promote the cessation of photoperiodic inhibition in does to anestrus and extend their reproductive activity.

It was verified, in December, in the peak of daylight, that non-experimental nulliparous goats hormonally induced to estrus could stimulate the sexual behavior and ovulation in 100% of the experimental nulliparous goats kept in the same pen (ratio 1:1) and in 50% of pluriparous and primiparous goats kept in nearby pens in the same barn. Such short stimulus was enough to maintain the cyclic state in almost all nulliparous goats and some adult goats from other pens until the end of study on mid-March. These findings highlighted the importance of the female effect on the stimulus of the sexual behavior and entry in the breeding season. In this context, Restall et al. (1995) also demonstrated that more than 75% of anestrus goats ovulated when the female ratio cyclic:anestrus was 3:10 and these animals were kept in a smaller pen of 100 m^2. Thus, an effective social stimulus for goats at anestrus time can come from their maintenance with a considerable number of cyclic females expressing sexual behavior into a relatively limited space, maximizing social interactions.

Our current results suggested the contributions of photoperiod, endogenous circadian rhythm, and other external factors on the control of seasonal reproduction in Saanen goats, maintained under tropical conditions, should be reevaluated. Likewise, a number of scientific conjunctures that refer to natural biotechniques into the breeding management can be considered aiming to a constant milk production throughout the year.

In conclusion, primiparous and pluriparous goats showed reproductive season in the autumn and winter time while nulliparous goats remained cyclical in winter time. Regardless of the parity order, all animals exhibited a marked seasonal anestrus in the spring. Social interactions, in early summer, were able to reestablish, from distinct degrees, the cyclical state in goats. Likewise, environmental and weight indexes had low to moderate influence, respectively, on the reproductive seasonality in Saanen goats kept under tropical conditions.

Acknowledgments The authors thank Embrapa and FAPERJ for their financial support. FZB and NPRA are CNPq fellows.

Compliance with ethical standards

This study and procedures were approved by the Ethical Committee for Animal Use of the Universidade Federal Fluminense (protocol no. 807).

Conflicts of interest The authors declare that they have no conflict of interest.

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