



**SEASONAL VARIATION IN OVULATORY ACTIVITY OF NUBIAN,
ALPINE AND NUBIAN × CRIOLLO DOES UNDER TROPICAL
PHOTOPERIOD (22° N)**

**[VARIACIÓN ESTACIONAL DE LA ACTIVIDAD OVULATORIA DE
CABRAS NUBIA, ALPINA Y NUBIA × CRIOLLO EN CONDICIONES DE
FOTOPERIODO TROPICAL (22° N)]**

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SUMMARY

In the present study, seasonal variation in ovulatory activity of Nubian, Alpine and Criollo × Nubian goats in the semiarid region of central-northern Mexico (22° 14' N) was examined. The study was conducted under natural photoperiod and climate conditions during a whole year. Eight female goats per breed were grouped separately and exposed to visual, olfactory and audible signals of bucks. Blood samples were obtained twice per week and serum progesterone concentrations were determined. All goats presented a clear pattern of seasonal ovulatory activity based on serum progesterone profiles. Length of the ovulatory activity period did not differ between genotypes ($P > 0.10$), and had an average duration of 4.3 months. Nevertheless Criollo × Nubian goats presented greater individual variation in dates of onset and end as well as length of this period ($P < 0.05$). Results indicate that female goats of genotypes which differ in latitude of origin, express a similar restricted pattern of seasonal ovulatory activity when subjected to small annual changes in photoperiod, adequate nutrition and incomplete socio-sexual stimulus,

Key words: Goat breeds; Seasonal reproduction; Tropical photoperiod.

RESUMEN

En el presente estudio se evaluó la variación de la actividad reproductiva estacional de cabras Nubia, Alpina y Nubia × Criolla en la región semiárida del norte centro de México (22° 14' N). El trabajo se realizó en condiciones de fotoperiodo y clima naturales, durante un año. Las cabras ($n=8$) de cada raza (Alpina, Nubia y Nubia × Criollo) se alojaron en corrales separados y estuvieron expuestas a las señales visuales, olfativas y auditivas de machos. Se tomaron muestras de sangre dos veces por semana y se determinó la concentración sérica de progesterona. De acuerdo con los perfiles de progesterona, todas las cabras mostraron un patrón estacional definido en su actividad ovulatoria. La amplitud del periodo de actividad ovulatoria no difirió entre razas ($P > 0.10$), presentando una duración promedio de 4.3 meses. Sin embargo, las cabras Nubia × Criollo mostraron mayor variación individual en las fechas de inicio y finalización, así como en la amplitud de este periodo ($P < 0.05$). Se concluye que hembras caprinas de genotipos originados en diferentes latitudes expresan un patrón similar restringido de actividad ovulatoria estacional cuando son expuestas a cambios anuales reducidos en duración del fotoperiodo, nutrición adecuada y estimulación socio-sexual parcial.

Palabras clave: Razas caprinas; Reproducción estacional; Fotoperiodo tropical.

INTRODUCTION

Goat production in Mexico is practiced under tropical and subtropical latitudes with the Criollo goat (*Capra hircus hircus* L) as a highly used genotype. The Mexican Criollo goat has been repeatedly crossed, during the last 40 years, with tropical (Nubian) or temperate breeds (Alpine, Saanen and Toggenburg), as a way to increase productivity. Nevertheless, the Criollo genetic makeup still remains in a significant degree in several goat herds throughout the country (Duarte *et al.*, 2008). Herds with purebred Alpine, Saanen, Toggenburg and Nubian goats also exist.

Irrespective of diverse latitude and climatic conditions, several studies in Mexico have demonstrated a seasonal pattern regarding reproductive activity in the Criollo female goat. However, this seasonality has been shown to vary from almost non-seasonal (Valencia *et al.*, 1990) to a clearly defined seasonal pattern (Duarte *et al.*, 2008; Estrada *et al.*, 2009).

With respect to reproductive seasonality of purebred goats in the tropical and subtropical latitudes of Mexico, very few reports have been published. A rather weak seasonal effect on reproductive activity, inferred after analyzing the kidding pattern throughout the year has been observed in Nubian (Mellado *et al.*, 1991), as well as in Alpine goats (Silva *et al.*, 1998). Conceptions occurred all year round although more concentrated during a portion of the year. These results strongly contrast with the 8 to 9 month long anovulatory season found by Chemineau *et al.* (1992), in Alpine does subjected to a tropical photoperiod. However it coincides with the seasonal reproductive pattern observed by Amoah *et al.* (1996) in Nubian goats under subtropical latitude, and by Valencia *et al.* (1990) in Granadina goats in tropical conditions.

Reproduction in goats is considered to be an endogenous biological rhythm entrained by changes in photoperiod. However, nutritional status or body condition (Meza-Herrera *et al.*, 2007; Duarte *et al.*, 2008; Estrada *et al.*, 2009; Urrutia-Morales *et al.*, 2009; Flores-Najera *et al.*, 2010; Rosales-Nieto *et al.*, 2011), continuous presence of the male (Restall, 1992; Rincon *et al.*, 1999), and other environmental cues as rainfall (Silva *et al.*, 1998; Mellado *et al.*, 1991), could influence interactively the expression of this rhythm. Such interactive effect might gain importance when a weak photoperiodic signal is present as occurs in the tropical and subtropical latitudes (Chemineau *et al.*, 2004). In turn, this situation as well as differences in the expression of the annual reproductive rhythm in genotypes originated at distinct latitudes (Amoah *et al.*, 1996), have to be accounted when determining reproductive management strategies in goat herds located in tropical and subtropical regions.

The present study was conducted in order to contrast the seasonal pattern of ovulatory activity in genotypes of different origin (Nubian, Alpine, and Criollo × Nubian), maintained under tropical latitude (22° N) and exposed to natural climatic changes in controlled nutritional conditions.

MATERIAL AND METHODS

Animals and management

All the experimental procedures were performed according to the “International Guiding Principles for Biomedical Research Involving Animals” (available at: [http://www.cioms.ch/1985 texts of guidelines.htm](http://www.cioms.ch/1985%20texts%20of%20guidelines.htm)). The study was conducted at the Faculty of Agronomy Experimental Station of the San Luis Potosí Autonomous University, located in San Luis Potosí, Mexico, latitude 22° 14' N, longitude 100° 53' W, and 1,835 m above sea level. The photoperiod in this area varies from 13 h 22 min of light at the summer solstice to 10 h 38 min of light at the winter solstice and the climate is classified as dry. During the experiment average annual rainfall was 285.9 mm, with 66.8% of rainfall occurring between June and September. Average annual temperature was 17.5 °C, with an average maximum and minimum of 26.6 and 8.7 °C, respectively (Figure 1).

Non-pregnant, non-lactating adult Alpine, Nubian and Criollo × Nubian does (n=8 / genotype), were maintained for 12 months beginning in April under natural photoperiod and climate. Does of each breed were kept in separate open pens with shaded area, and fed to constantly fulfill the maintenance requirements (NRC, 1981). Fresh water and minerals were offered in a free access basis. Visual, olfactory and auditory contact between experimental does and bucks was allowed, but physical contact was not permitted.

Body weights (BW) were recorded at weekly intervals from May 15th until the end of the experiment and their individual variations were used to adjust feeding. Blood samples were collected twice a week by jugular venipuncture and kept at ambient temperature for 5 h until clotting. Serum was then obtained after centrifugation at 2500 g for 15 min and stored at -20°C until progesterone was determined by a solid phase radioimmunoassay (Coat-a-Count; DPC, Los Angeles, CA, USA). Sensitivity of the assay was 0.03 ng ml⁻¹ and intra- and inter-assay coefficients of variation were 4.5 and 8.5 %, respectively.

Definitions

Ovulation was considered to have occurred 3 days before serum progesterone concentrations increased to 1.0 ng/ml or beyond in at least 3 consecutive samples. According to this criterion distinct ovulatory an

anovulatory stages were observed in the 3 genotypes analyzed. Individual onset and end of cyclic ovulatory activity (ending = no ovulations registered for ≥ 36 days) as well as length of ovulatory cycles (number of days between 2 consecutive ovulations) were estimated from serum progesterone profiles. Mean duration of cyclic ovulatory activity was defined as the number of days between the first and last registered ovulations within the experimental period. Ovulatory cycles were classified according to their length as short (<17 days), normal (17-25 days) or long (>25 days) cycles.

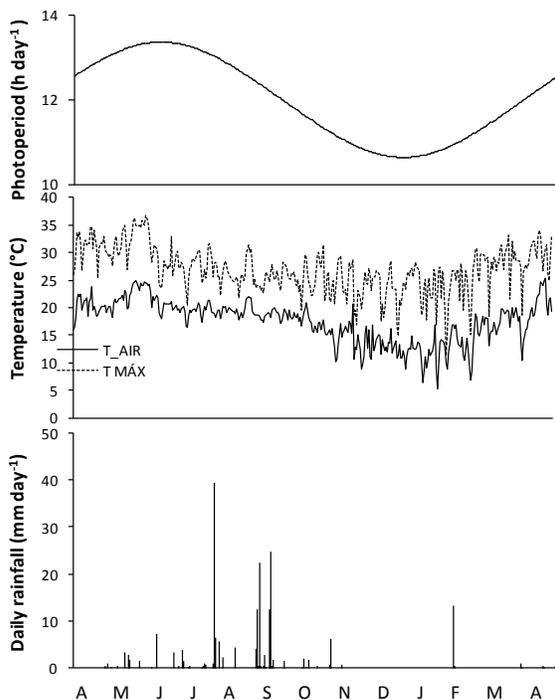


Figure 1. Daily changes in photoperiod, ambient temperature and rainfall during the experimental period.

Statistical analysis

Statistical analysis was performed with the SAS statistical software (SAS Inst. Inc., Cary NC, USA, 2006). BW changes with respect to initial BW and length of ovulatory cycles were analyzed by an ANOVA for a repeated measures design (genotype as between, and time/cycle # and genotype \times time/cycle # as within subject effects). Time of onset, time of ending, and length of cyclic ovulatory activity were compared between genotypes through ANOVA for a completely randomized design. Variances of these last responses were compared with Fisher's Test of the Equality of Two Variances (Snedecor and Cochran, 1989). Chi square or Fisher exact tests were used, as

appropriate, to compare proportions in frequency variables.

RESULTS

Body weight change

Initial and final BW's did not differ ($P > 0.05$) among genotypes (35.06 , 39.12 and 42.31 ± 3.03 kg, and 38.9 , 42.8 and 44.4 ± 2.6 kg in Alpine, Nubian and Criollo \times Nubian does, respectively). However, changes of BW with respect to initial BW were influenced by time and the interaction of genotype \times time ($P < 0.01$), but not by genotype as a main effect ($P > 0.05$). Although BW variations across time presented slight differences among genotypes, the long term profile of this variation was directed toward an increase in BW in the 3 ones evaluated (Figure 2).

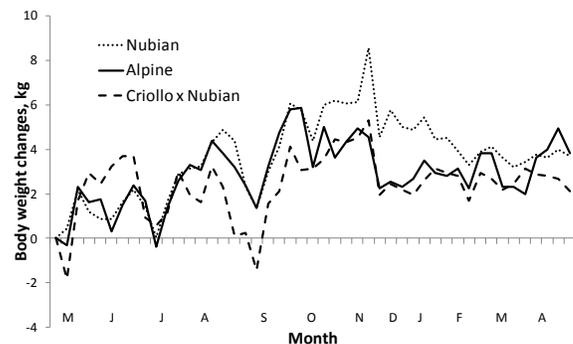


Figure 2. Body weight changes throughout the experimental period in Alpine, Nubian and Criollo \times Nubian female goats maintained under tropical latitude (22° N), natural climate and controlled nutrition.

Ovulatory activity

During the course of the 12 month experimental period, and according to serum progesterone profiles, a clear seasonal pattern of ovulatory activity became apparent in the 3 genotypes evaluated (Fig. 3). 45.8 % of the goats experienced at least one short- increase in serum progesterone concentrations (≥ 1.0 ng/ml of progesterone in only one sample) before the first recorded ovulation. The percentage of does experiencing these short- increases in progesterone was greater ($P = 0.07$) in the Criollo \times Nubian (75 %) as compared to the Nubian (25 %) genotype. No differences in this response were found when considering the other genotype comparisons (Nubian vs. Alpine and Nubian vs. Criollo \times Nubian). Once initiating cyclic ovulatory activity and before its end, 7 does (29.1%) presented other short-increases in serum progesterone (1, 2 and 4 does in the Nubian, Alpine and Criollo \times Nubian genotype, respectively; $P > 0.10$).

Two does (one in the Alpine and one in the Criollo × Nubian genotype; $P > 0.10$) experienced short-term increases in serum progesterone after the ending of cyclic ovulatory activity as well. The first and last registered ovulations within the experimental period occurred in two different Criollo × Nubian does (August 25 and March 2, respectively).

Cyclic ovulatory activity

Dates of onset and end of cyclic ovulatory activity, as well as its length, were similar ($P > 0.10$) among genotypes (Table 1). However, within group variability of these responses was greater ($P < 0.05$) in the Criollo × Nubian as compared to the Alpine genotype, and tended to be greater (estimated F value= 3.2; significant F value at $P < 0.05 = 3.79$), for the date of ending and length of cyclic ovulatory activity when compared to the Nubian genotype. No differences between the Alpine and Nubian genotypes were detected within group variability of cyclic ovulatory activity descriptors. Mean dates of the onset and end of cyclic ovulatory activity were September 28, and February 5, and general mean length of cyclic ovulatory activity period was 129.8 days.

Ovulatory cycles

The percentage of ovulatory cycles which were normal in length differed between the 3 genotypes evaluated (Table 2). The Criollo × Nubian genotype presented

fewer normal length ovulatory cycles as compared to the Nubian ($P = 0.09$) and Alpine ($P < 0.01$) genotypes. Normal length ovulatory cycles were also less in the Nubian vs. Alpine genotype ($P < 0.05$). None short ovulatory cycles (<17 days) were found during the experimental period in the 3 genotypes evaluated.

Mean length of ovulatory cycles did not differ ($P > 0.10$) between genotypes (23.1, 21.8 and 25.2 days in Alpine, Nubian and Criollo × Nubian, respectively).

DISCUSION

Differences in the beginning and ending of the reproductive season detected among breeds (Amoah *et al.*, 1996) reflect differences in synchronization rhythm due to different photoperiod present in their native locations (O’Callaghan *et al.*, 1992). The aim of this study was to determine if under tropical latitude, natural climate, and controlled nutritional conditions, those differences in their seasonal reproductive rhythm are expressed. Results demonstrate that female goats kept under tropical photoperiod (22°N) display a marked seasonal variation in ovulatory activity. In addition, amplitude of the breeding season was similar on female goats of the Nubian, Alpine and Criollo × Nubian genotypes, with similar mean date of onset and end of ovulatory activity.

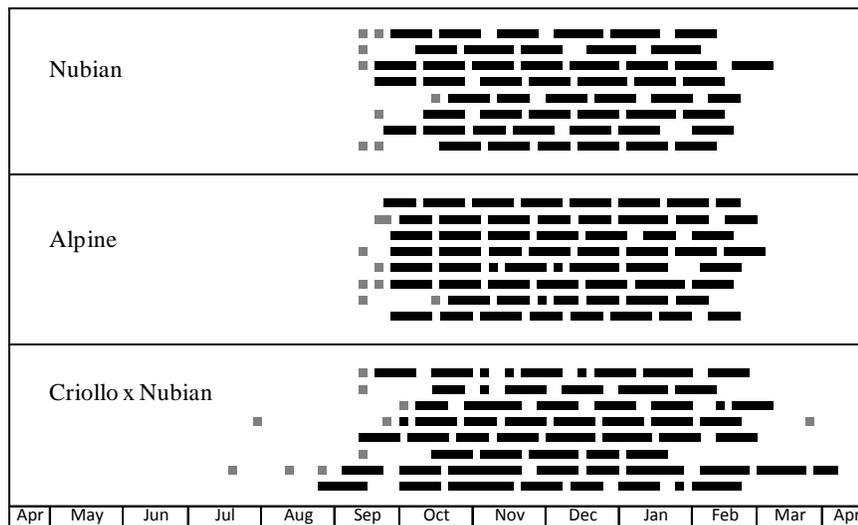


Figure 3. Individual ovulatory activity in Alpine, Nubian and Criollo × Nubian female goats maintained under tropical latitude (22 ° N), natural climate and controlled nutrition. Ovulatory activity was discerned from twice-weekly determinations of progesterone by radioimmunoassay. One line represents one female goat. A closed square or rectangle represents one or more than one progesterone measurements above 1 ng/ml of serum, and are considered as indicative of at least one luteal structure or corpus luteum, respectively.

Table 1. Mean dates \pm S.D. for onset and end, and mean length of cyclic ovulatory activity in Alpine, Nubian and Criollo \times Nubian female goats maintained under subtropical latitude (22° N), natural climate and controlled nutrition.

| Cyclic ovulatory activity | Alpine | Nubian | Criollo \times Nubian |
|---------------------------|----------------------------------|-----------------------------------|-----------------------------------|
| Onset | Sep 28th \pm 8.99 ^a | Oct 3rd \pm 12.90 ^{ab} | Sep 23rd \pm 18.93 ^b |
| End | Feb 8th \pm 7.88 ^a | Feb 1st \pm 9.58 ^{ab} | Feb 6th \pm 17.20 ^b |
| Length | 132.9 \pm 15.31 ^a | 120.4 \pm 17.33 ^{ab} | 136.1 \pm 31.03 ^b |

Genotype effect, $P > 0.10$;

^{a, b} Different superscripts within the same line indicate variance differences ($P < 0.05$).

Table 2. Number and percentage (%) of ovulatory cycles according to their length in Nubian, Alpine and Criollo \times Nubian female goats maintained under subtropical latitude (22° N), natural climate and controlled nutrition.

| Genotype | No. of cycles | Cycle Length (days) | | |
|-------------------------|---------------|---------------------|--------------|-----------|
| | | Short <17 | Normal 17-25 | Long > 25 |
| Alpine | 29 | 0 (0) | 29 (100) | 0 (0) |
| Nubian | 33 | 0 (0) | 29 (87.9) | 4 (12.1) |
| Criollo \times Nubian | 27 | 0 (0) | 19 (70.4) | 8 (29.6) |
| Total | 89 | 0 (0) | 77 (86.5) | 12 (13.5) |

Normal and long cycles: Alpine vs. Nubian, $P < 0.05$; Alpine vs. Criollo \times Nubian, $P < 0.01$; Nubian vs. Criollo \times Nubian, $P = 0.09$

Although, in the present study two contrasting reproductive seasonal breeds were included, one of them recognized to show a large breeding season (Nubian), and the other characterized to show a very short breeding season (Alpine) (Amoah et al., 1996), those differences among breeds were not expressed. It is known that nocturnal melatonin secretion is under strong genetic influence in ewes (Zarazaga et al., 1998; Chemineau et al., 2002), suggesting that different response in female goats to photoperiod is also under genetic influence too (Chemineau et al., 2004). This may help to explain the differences in the reproductive seasonality previously observed among breeds. However, in the present study, that genetic difference was not observed.

A clear seasonal pattern in cyclic ovulatory activity was observed in the 3 genotypes evaluated, with mean dates of onset and end, and length that did not differ (Table 1, $P > 0.10$). Chemineau et al. (1992) reported very similar results in Alpine does maintained at temperate latitude (47° 25' N), irrespective to the photoperiodic regime to which they were subjected (tropical, 11 to 13 h or temperate, 8 to 16 h of light from winter to summer solstices). Averages of the onset, end and length of ovulatory season reported by Chemineau et al. (1992) were early October, early February and 148 days, respectively. A coincident pattern of seasonal ovulatory and breeding activity was found by Valencia et al. (1990) in Granadina goats under tropical latitude and by Amoah et al. (1996) in Alpine does under subtropical conditions. However, Amoah et al. (1996) registered an increased breeding

season in Nubian does, which was twice in length (8 to 11 mo) than the ovulatory season observed in the Nubian genotype in the present study (120.4 days, Table 1). Moreover, Mellado et al. (1991) observed conceptions all year round in Nubian goats under subtropical latitude, with only a moderate depression in fertility rate (-20 %) during February and March. Silva et al. (1998), also found an extended breeding season (\approx 8 mo) in Alpine goats under tropical latitude, with occasional conceptions during the non-breeding season as well.

Tropical Creole goats in the Guadeloupe Island, are considered to be almost non-seasonal breeders under their native tropical conditions (Chemineau, 1986). A similar situation occurs when this genotype is subjected to a simulated tropical photoperiod in a temperate climate, however, a defined seasonal pattern in ovulatory activity was observed when subjected to both temperate climate and photoperiodic regime (Chemineau et al., 1992). The Mexican Criollo goat is not considered to be totally of tropical origin since it was derived from the Spanish breeds Granadina, Murciana, and Malagueña (raised close to the subtropical region and derived from the Pyrenean goat), as well as from the Blanca Celtiberica which was introduced to Spain from tropical Africa (Sudan). Nonetheless, all of these breeds are considered to be non-seasonal breeders in the Spanish regions in which they are raised. In other study (December to July), and under controlled nutritional conditions Valencia et al. (1990) found a monthly percentage of does ovulating above 50 % in Mexican Criollo goats except in March,

April and May (30, 10 and 21 %, respectively). They concluded that this genotype expresses a weak seasonality in ovulatory activity under tropical latitude when nutritional conditions are adequate. A similar extended ovulatory season was found by Rivera *et al.* (2003) in Criollo goats of Argentina (8 mo with > 50 % of does ovulating/month). In contrast, Duarte *et al.* (2008) and Estrada *et al.* (2009) reported different seasons of ovulatory and anovulatory activity in Mexican Criollo does independently of their nutritional status (mean length for ovulatory and anovulatory stages \approx 145 and 220 days, respectively).

Seasonal reproduction in goats is considered to be an endogenous biological rhythm entrained by changes in photoperiod. However, when changes in photoperiod represent a “weak” signal, as it happens in tropical and subtropical latitudes, other environmental clues might be used as complements to fine-tune the expression of the rhythm (Chemineau *et al.*, 2004). Nutrition (Duarte *et al.*, 2008; Estrada *et al.*, 2009), continuous presence of the male (Restall, 1992; Urrutia *et al.*, 2008; Rincon *et al.*, 1999), and climatic aspects like rainfall (Mellado *et al.*, 1991; Silva *et al.*, 1998), appear to be important components of these environmental clues. The interactive effect of such factors might explain the large variability observed when characterizing the seasonal pattern of reproductive activity in female goats under tropical and subtropical conditions.

In the present study, rations were assigned in an individual body weight basis to constantly fulfill maintenance requirements. In turn, slight increases in body weight throughout the experimental period were observed, which followed a similar pattern in the 3 experimental groups (Fig. 2). Moreover, irrespective to genotype, a similar degree of visual, olfactory and auditory contact between does and bucks was allowed throughout the entire experimental period. Accordingly, it can be concluded that neither nutritional status nor degree of contact with males were factors biasing the comparison of seasonal ovulatory activity between the evaluated genotypes.

Restall (1992), suggested that the annual reproductive rhythm in goats consists of three periods: an active and responsive period which together constitute the breeding season, and the quiescent period which corresponds to the non-breeding or anovulatory season. During the responsive period, does may or may not ovulate depending on the quality of non-photoperiodic external stimuli they receive, mainly those associated to interaction with the male. In the active and quiescent period, spontaneous ovulations and anovulation occur. Corteel (1977) introduced a similar concept to describe the non-breeding season in temperate goat breeds, which included a deep anoestrus period with no ovulations, and a transition period to the breeding season in which ovulations can

be induced through the male effect. In the present study, although males were close to the experimental does, no physical contact between them was allowed. In such conditions, the experimental does probably were not able to express the responsive (or transition) period of their annual reproductive cycle, and only the so called active period was registered. In turn, this could be behind the observed lack of difference between the patterns of seasonal ovulatory activity in the evaluated genotypes. In addition, it might help to explain the contrasting shortened length of the ovulatory season observed in this study and by other researchers (Duarte *et al.*, 2008; Estrada *et al.*, 2009), as opposed to the extended breeding season found in does maintained under natural conditions and exposed to males (Mellado *et al.*, 1991; Amoah *et al.*, 1996; Silva *et al.*, 1998).

An interesting finding in this study is the high variability in expression of seasonal cyclic ovulatory activity (Table 1), and proportion of estrous cycle irregularities (Table 2 and Figure 3) observed in the Criollo \times Nubian group. The Nubian genotype appeared to be intermediate in this respect. Chemineau *et al.* (1992) also observed a greater variability in the cyclic ovulatory activity pattern and a greater proportion of non-regular estrous cycles in tropical Creole does when a simulated tropical vs. temperate photoperiodic regime was applied. Does in the temperate photoperiod group did manifest a marked but extended season of ovulatory activity, while those subjected to the tropical photoperiodic regime demonstrated much less seasonality. In an individual basis, most of the females in the tropical photoperiod group presented anovular periods of variable length, but with a lack of synchrony as a group. A similar non-synchronized ovulatory/anovulatory pattern was manifested in a group of pinealectomized ewes (Barrell *et al.*, 2000), which due to pinealectomy are supposed to have a disruption in the internal chemical transduction of the photoperiodic signal, and the disabling of this signal as an external synchronizer (Malpaux *et al.*, 2001). Accordingly, the asynchrony between does in the tropical photoperiod group of Chemineau *et al.* (1992) study, might be reflecting the existence of a weak external synchronizing signal for the endogenous rhythm of reproductive activity (tropical photoperiodic regime), and/or a weak link with this external synchronizer due to genotype origin (tropical origin of the Guadeloupean Creole goats). In regard to findings in the present study, it is difficult to discern the implications of the high variance and irregularities in the ovulatory activity pattern observed within the Criollo \times Nubian group. However, it is tempting to speculate that it could be a marker of a “weakened” role for the photoperiodic signal as external synchronizer of the annual reproductive rhythm. In turn, this might imply that other external signals have to be used as clues to determine the

pattern of this rhythm, and probably, that the responsive period within it would be broader. Addressing of these issues in future research would be worth if controlled reproductive management for goats in tropical and subtropical latitudes is the goal.

CONCLUSIONS

Independently of genotype origin and under tropical latitude and controlled nutritional conditions, female goats in the present study presented a trend of similar distinct and shortened period of cyclic ovulatory activity within the year long experimental period. Implications of the high variability in the cyclic ovulatory activity pattern and proportion of estrous cycle irregularities observed in the Criollo × Nubian does is difficult to discern as well as potential effect on the observed ovulatory responses associated to the incomplete socio-sexual stimulus that was provided (no physical contact with the males).

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