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Reproductive response of synchronized and extensively grazed Blackbelly ewes during the summer in the tropics

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Abstract The present experiment aimed to evaluate the reproductive response of Blackbelly ewes synchronized with low doses of eCG during the summer in a tropical climate. Sixty multiparous hair ewes were divided into three groups (20 ewes/group): control group (CG; 0 IU of eCG), treated group 1 (TG-1; 140 IU of eCG) and treated group 2 (TG-2; 300 IU of eCG). The study found that the three groups of ewes showed differences in their reproductive behavior ($P < 0.01$). Indeed, all the ewes in the TG-1 and TG-2 groups presented a higher response to estrus than the CG ($P < 0.01$). In the same way, TG-1 and TG-2 had an onset to estrus in less time ($P < 0.01$). On the other hand, prolificacy was also higher in TG-1 and TG-2 ($P < 0.05$). Likewise, ewes from TG-1 and TG-2 had more lambs than those from CG ($P < 0.05$). On the other hand, the variables of fertility and gestation length were similar regardless of the group of ewes ($P > 0.05$). It´s concluded that the ewes treated with eCG presented good reproductive behavior, and the majority did so in the first 24 to 36 h after removing the intravaginal device.

Keywords: estrous cycle, reproductive behavior, temperature and humidity index, weight and body condition

1. Introduction

Globally, livestock production is not enough to meet the demand for the protein of animal origin for human consumption; by the year 2050, it´s expected that it will be necessary to increase production by more than 60 % (Alexandratos and Bruinsma 2012). In this context, the FAO projected that by 2022 the share of sheep meat would increase by 80 % (FAO 2014). Among the countries with the highest production of ewes meat are China, with 2 million tons, and Australia and New Zealand, with 500,000 tons, respectively (FAOSTAT 2015). For its part, Mexico contributes 117,862 tons with 8,683,835 heads of sheep (SIAP 2016, 2018). In the country, the states with the highest number of sheep are the state of Mexico (1,371,356), Hidalgo (1,161,183), and Veracruz (698,520); the state of Guerrero has 150,263 heads and produces 2,595 tons of sheep meat (SIAP 2018). It is evident that, despite the increase (84 %) in the national production of sheep meat, there is a deficit (16 %) to satisfy local consumption; exporting 10,701,868 tons from countries such as New Zealand, the United States, Australia, Chile and Uruguay (CNOG 2012).

In sheep herds in Mexico, where extensive grazing is managed with night confinement, the animals consume native grass and seasonal fruits; generally, these sheep have a person (shepherd) who accompanies them during grazing (Hernández-Ruiz et al 2021). Under this management, the male always accompanies the females and covers them at the time they are in estrus; therefore, it is not possible to control that the females gestate at an optimal time so, when the young are born there is food available, which strongly affects pregnant females causing poor nutrition, due to the conditions in which they develop (Laporte-Broux et al 2011; Hernández-Ruiz et al 2021). An alternative to solve this problem and increase the productivity of existing herds is the implementation of reproductive programs that include estrus synchronization protocols with synthetic hormones, progestogens, and equine chorionic gonadotropin (eCG). These protocols are very efficient in inducing and synchronizing estrus in small ruminants and avoiding contact with the male at all times (Macías-Cruz et al 2012). Indeed, Macías-Cruz et al (2013) synchronized ewes with intravaginal sponges, and 24 h before removing the devices, they applied a dose of 140 and 280 IU of eCG intramuscularly. These authors found that 100 % of the ewes responded to estrus. For their part, García y González et al (2018) synchronized with CIDR and eCG, and found that 100 % of the ewes responded to treatment. This practice ensures that oestrus and parturitions are concentrated in specific times for the producer that coincide when there is greater availability of forage, a management that allows optimizing the reproduction of sheep herds (Rosado et al 1998; Macías-Cruz et al 2012). Some factors influence the efficiency of synchronization protocols, such as nutrition, reproductive

season, rainfall, forage availability, intensity of sexual behavior, and temperature and humidity index (THI).

The sexual behavior of rams and bucks is important for the sexual stimulation of females. For example, anestric ewes and goats stimulated by a sexually active male are induced into estrus (Ponce et al 2014; Calderón-Leyva et al 2018). Males display intense sexual behavior when exposed to ewes and goats in heat, previously synchronized with progestogens and eCG (Giriboni et al 2017; Abecia et al 2020). On the other hand, another factor that influences the efficiency of the synchronization protocols is the THI; in heat-stressed ewes, their sexual behavior and the quality of the corpus luteum are affected (Gastelum-Delgado et al 2015; Macías-Cruz et al 2015). Therefore, the present study aimed to evaluate the reproductive response of Blackbelly ewes synchronized with low doses of eCG, as well as the sexual behavior of rams exposed to females during the summer in tropical weather.

2. Material and Methods

2.1. General

The present study was conducted in June 2019 in a flock of hair ewes in San Jeronimo de Juarez, Benito Juarez municipality, Guerrero, Mexico. The region is part of the Costa Grande of the state and is located in the country's tropics (geographical coordinates: 17°08'09"N and 100°28'08"W). The average annual temperatures during the summer are 32 °C and during the winter 21 °C (García 1987).

2.2. Animals and synchronization protocol

For the study, 60 multiparous Blackbelly ewes were used, which were divided into three groups (20 ewes/group): control group (CG; 0 IU of eCG), treated group 1 (TG-1; 140 IU of eCG) and treated group 2 (TG-2; 300 IU of eCG), balanced according to weight (34 ± 5.5) and body condition (1.7 ± 0.5). All ewes underwent an estrus synchronization protocol with flurogestone acetate (FGA; Chronogest, Intervet) and equine chorionic gonadotropin (eCG). The protocol consisted of placing an intravaginal sponge impregnated with 20 mg of FGA for 10 d, and 24 h before removing the sponges; an intramuscular injection of 0, 140, or 300 IU of eCG was applied. Estrus and natural mating were detected with three rams of the Blackbelly genotype between 12 and 48 h after the synchronization protocol ended. When the ewes accepted the first mount, it was considered in estrus, and a second mount was given 12 h later. The ewes that presented estrus were recorded, as well as the time. On the other hand, sexual behavior was measured for 3 d; for this, the ewes were locked in pens where the males were introduced after they were taken to graze with the rest of the herd.

2.3. Study variables

2.3.1. Ewes

In the study, reproductive variables of the female were evaluated, such as response to estrus (percentage of ewes in estrus of the total treated), onset to estrus (time interval between the completion of the protocol and the appearance of signs of estrus), fertility (percentage of treated ewes that gave birth), prolificacy (number of lambs born per ewes that gave birth), single lambing (percentage of ewes with one lamb at birth out of the total that gave birth), multiple lambing (percentage of ewes with two or more lambs at birth out of the total that gave birth) and length of gestation (days elapsed from mating to parturition).

2.3.2. Rams

In males, sexual behavior was evaluated in each group of females (CG, GT-1, and TG-2). Appetitive sexual behaviors (nudging, pawing, flehmen, anogenital sniffing, vocalizations, and self-urination) and consummatory sexual behaviors (mounting attempts, mounting without intromission, and mounting with intromission).

2.4. Climatic variables

The climatic information was requested from the meteorological station number 12,161 (Atoyac de Alvarez, Guerrero), where the environmental temperature (°C) and relative humidity (%) were provided, which was recorded every ten minutes for 24 h. With the above information, the temperature-humidity index (THI) was calculated with the equation proposed for ewes (Kelly and Bond 1971):

$$THI = T - \{[0,55*(1-RH)] * (T-14,4)\}$$

where T represents the ambient temperature and RH the relative humidity in decimals during the morning and afternoon.

2.5. Pre-experimental management and feeding

Before starting the experimental phase, the ewes were vitaminized (A, D, E, and complex B), dewormed (Ivermectin), and hoofless. The feeding of the females before and during the experimental period was based on grazing for 6 h (12:00 to 6:00 h) in paddocks that are used for growing mango and coconut palm trees. In addition, these animals consume native grass, tree leaves, and seasonal fruits such as mango and coconut. The ewes in a normal way and during the study period did not receive food supplementation. For their part, the males were brought to the females' pen only during the mating period.

2.6. Statistical analysis

The information was analyzed with the statistical program SAS (2004) under a completely randomized design. An analysis of variance was performed for the variables time to estrus, prolificacy, and gestation length using the GLM procedure, while the response to estrus, fertility, and percentages of single and multiple lambing were analyzed using the CATMOD procedure. Means were compared using Tukey's test for continuous variables and the $\chi 2$ test for categorical variables. Between-group differences in rams' sexual behavior were analyzed by comparing the frequencies of the behaviors observed in each group with a random

repartition, using Fisher's exact probability tests. The results are presented in percentage and mean ± SEM.

3. Results

3.1. Ewes reproductive behavior

The study found that the three groups of ewes showed differences in their reproductive performance ($P < 0.01$). Indeed, all the ewes in the TG-1 and TG-2 groups presented a high response to estrus (100 %) compared to the CG (60 %) ($P < 0.01$). In the same way, TG-1 and TG-2 began estrus in less time (25.55 ± 1.82 and 25.36 ± 1.82) ($P < 0.01$). On the other hand, prolificacy was also higher in TG-1 and TG-2 (2.00 ± 0.26 and 2.30 ± 0.26) ($P < 0.05$). Likewise, ewes from TG-1 and TG-2 had more lambs than those from CG ($P < 0.05$). On the other hand, fertility was higher in ewes treated with eCG than in those that did not receive the treatment ($P < 0.05$) (Table 1). All 60 eCG-treated ewes in the study responded to estrus within the first 48 h of observation (Figure 1). Response to estrus in the first 24 h after device removal was greater in GT-2 than in GT-1 and CG ($P < 0.05$). In contrast, in the first 24 to 36 h after removing the device, it was higher in TG-1 ($P < 0.05$) and similar in TG-2 and CG ($P > 0.05$). Finally, at 36 h after sponge removal, CG was higher than TG-1 and TG-2 ($P < 0.05$).

Table 1 Effect of eCG dose on reproductive efficiency of Blackbelly ewes.

Variable	Ewes groups		
	CG	TG-1	TG-2
Treated ewes, N	20	20	20
Lambing ewes, n	8	12	13
Response to estrus, %	60[b]	100[a]	100[a]
Onset to estrus, h	36.41 ± 2.2[b]	25.55 ± 1.82[a]	25.36 ± 1.82[a]
Fertility, %	40[b]	60[a]	65[a]
Prolificacy	1.1 ± 0.35[b]	2.00 ± 0.26[a]	2.30 ± 0.26[a]
Single lambing, %	75[b]	17[a]	15[a]
Multiple lambing, %	25[b]	83[a]	77[a]
Gestation length, d	150 ± 26[a]	150.25 ± 1.13[a]	149.00 ± 1.13[a]

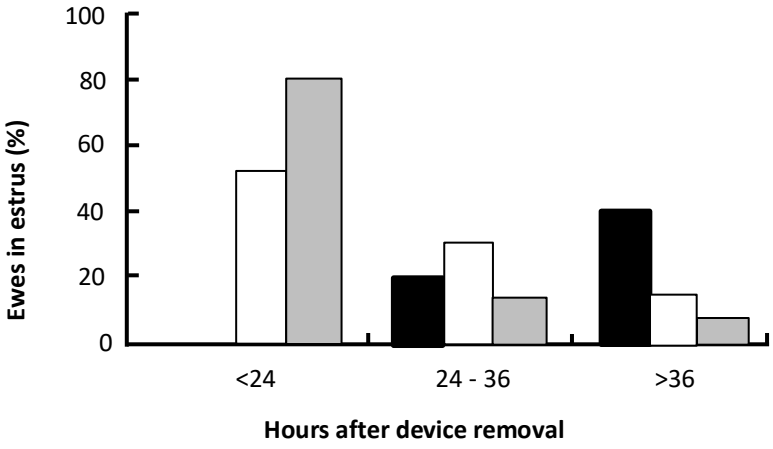

Figure 1 Distribution of estrus of ewes synchronized with progestogens and eCG. Black bars indicate CG, white bars TG-1, and gray bars TG-2.

3.2. Sexual behavior of rams

The males of the three groups showed intense sexual appetitive and consummatory behavior during the study ($P < 0.001$). Indeed, the males of the CG had a greater display of sexual behaviors nudging and pawing, than the males of the TG-1 and TG-2 ($P < 0.001$). Likewise, the males of the CG had

a greater number of anogenital sniffing than the males of the TG-1 and TG-2 ($P < 0.05$). In contrast, the rest of the appetitive sexual behavior variables were similar between groups of males (flehmen, vocalizations, and self-urination) ($P > 0.05$) [(Figure 2; appetitive sexual behavior (A)]. On the other hand, it was observed that males exposed to TG-1 and TG-2 ewes had a greater number of nudging in the first two days of contact ($P < 0.001$). In contrast, on day 3 of contact with females, the number of nudging decreased to increase the number of mountings without and with intromission ($P < 0.001$) [(Figure 2; consummatory sexual behavior (B)].

3.3. Environmental conditions

THI results ranged from 61 to 90 U (Figure 3). THI of 80 U in the morning and 90 U in the afternoon were obtained. This evidences the presence of severe heat stress in sheep from the desert (Marai et al 2007) and tropical regions (Ruiz-Ortega et al 2022).

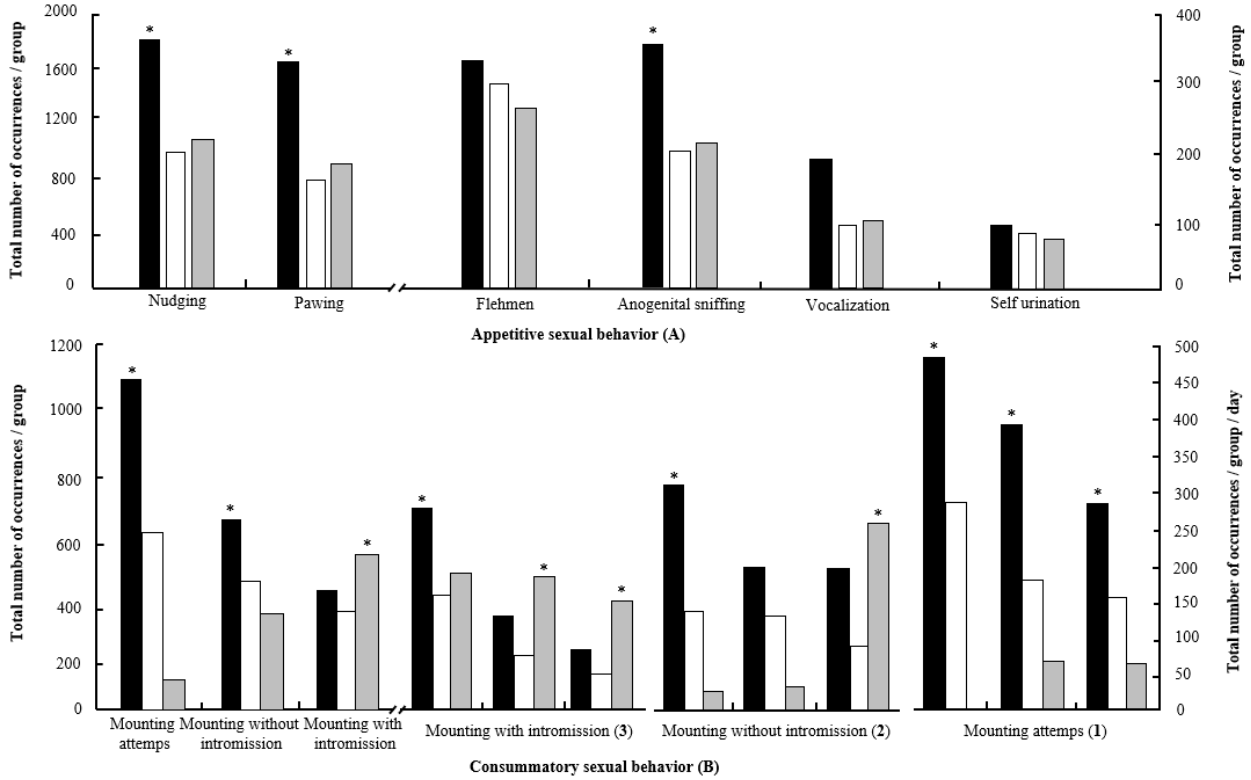

Figure 2 Cumulative frequencies of the sexual behaviors displayed by the rams during the experiment, appetitive sexual behavior (A): nudging, pawing, flehmen, anogenital sniffing, vocalizations, and self-urination. Consummatory sexual behavior (B): mounting attempts, mounting without intromission, and mounting with intromission. In figure B, the right axis is the number of occurrences per day (1, 2 and 3), on the left axis is the total of the three days. Black bars represent CG, white bars GT-1, and gray bars TG-2. *Denotes significant difference within sexual behavior by group ($P < 0.001$).

4. Discussion

The present study's data show that the ewes treated with eCG had a greater reproductive response than those of the group to which the gonadotropin was not applied. On the other hand, the rams had greater appetitive sexual behavior in the CG and greater consummatory sexual behavior in the ewes of the groups TG-1 and TG-2. Finally, the Guerrero tropics' local environmental conditions (THI) indicate severe heat stress in ewes.

4.1. Ewes' sexual behavior

Previous studies have shown that ewes treated with progestogen and a dose of eCG (> 300 IU) have a higher response to estrus (> 90 %) than those that were not treated with gonadotropin (Macías-Cruz et al 2009; López-García et al 2021). However, it has been suggested that doses of less than 300 IU of eCG are not enough to stimulate follicular development and a reduced response to estrus in ewes (Romano et al 1996). In this regard, Macías-Cruz et al (2013) mention that ewes synchronized with doses of 140 and 300 IU of eCG is sufficient to stimulate the estrous response in hot environmental conditions in northwestern Mexico. The present study was conducted under tropical conditions during the summer, with adverse environmental conditions (relative humidity and maximum environmental temperature of 90% and 36.8 °C, respectively) (Habeeb et al 2018). These environmental features strongly affect reproductive behavior in domestic animals. Regarding estrus distribution, most of the ewes treated with doses of 300 IU of eCG presented estrus in the first 24 h after removing the intravaginal device. These results are probably because with a higher dose of gonadotropin, and there are greater follicular development and release of estradiol (Ben Säid et al 2007). For example, Quintero-Elisea et al (2011) worked with doses of 100, 200,

and 400 IU of eCG and observed that the ewes at a higher dose of gonadotropin presented estrus in less time. In the present study, a similar time was observed with doses of 140 and 300 IU of gonadotropin compared to the group that did not receive the hormone. On the other hand, ewes from TG-1 and TG-2 that were treated with eCG had similar prolificacy compared to CG. Indeed, some studies mention that it´s necessary for ewes synchronized with progestogens to receive a minimum dose of eCG to stimulate follicular development and ovulation, increasing prolificacy (Ben Säid et al 2007; Macías-Cruz et al 2009; Kermani et al 2012). In the present study, fertility was affected in ewes treated with gonadotropin compared to the untreated group. Consistent with these results, Luther et al (2007) reported that ewes treated with eCG increased the pregnancy rate. For his part, Ali (2007) observed that treatment with eCG in hair ewes did not increase the pregnancy rate. Knowing this information is important since it shows that the decrease in the dose of eCG in ewes is effective since it does not affect reproductive performance. In general, the present study was conducted in a tropical region where the environmental conditions are heat stress, and the ewes were not affected in their reproductive performance. However, it is necessary to evaluate follicular development in these ewes under heat-stress conditions in the tropics of Guerrero, Mexico.

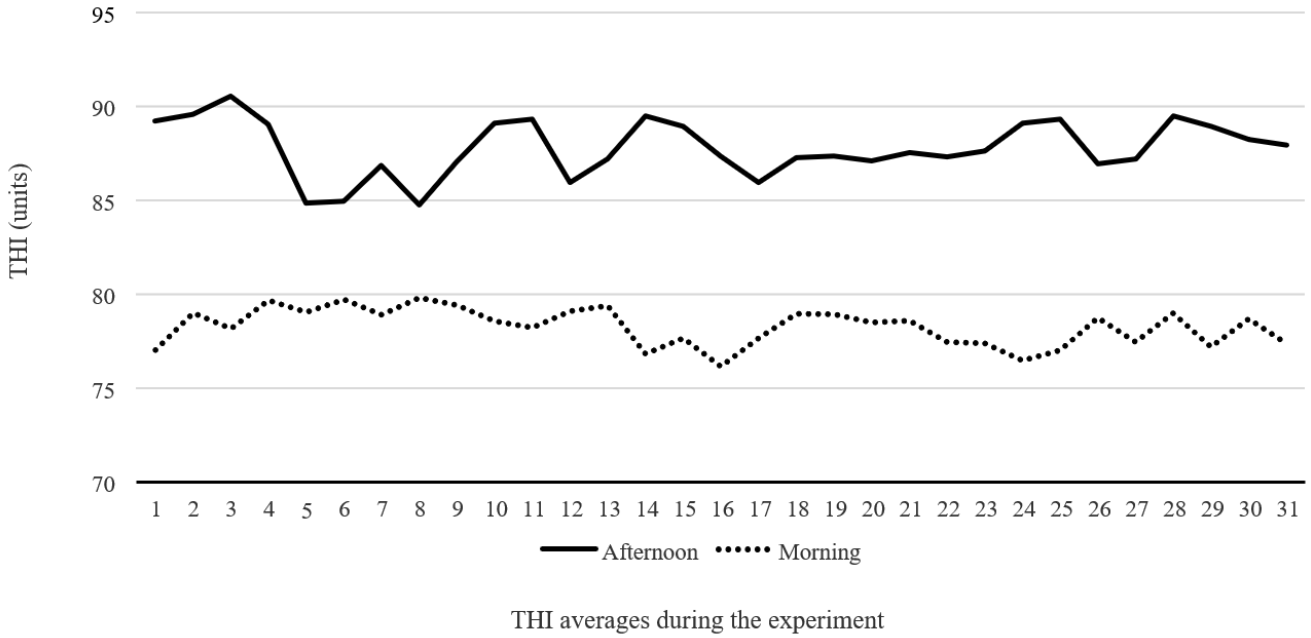

Figure 3 Temperature and humidity index (THI) adjusted for wind speed and radiation in the morning and night during the experimental period.

4.2. Sexual behavior of rams

In rams, appetitive sexual behavior implies a male's characteristic display or ritual directing actions to a female who expects to mate without this happening. Instead, consummatory sexual behavior consists of sexual contact with the female that ends with copulation (Ball and Balthazart 2008). The present study found that the males of the CG presented a greater appetitive sexual behavior. Indeed, rams exposed to anovulatory ewes exhibit intense sexual behavior (nudging, pawing, anogenital sniffing) (Calderón-Leyva et al 2018; González-Tavizón et al 2022). During the male effect, goats placed in contact with seasonal anestric goats show intense appetitive sexual behavior (e. g., nudging, anogenital sniffing, vocalizations) (Ponce et al 2014; Hernández-Ruiz et al 2021). In the present study, it was found that males exposed to ewes from the CG presented greater anogenital sniffing. Physical contact with the ewes is important to stimulate the perianal region of the female since, in this area, there is a large number of sweat glands, and there is a high concentration of pheromonal behavior that stimulates sniffing in this area and consequently stimulate the expression of flehmen (Blissitt et al 1990).

Interestingly, in the present study, there was no effect of flehmen behavior between groups of males; this was probably because the ewes were not in estrus when it occurred. This situation confirms the sexual response capacity of the ewes, a behavior that increases the male's libido to perform other sexual behaviors during courtship and copulation (González-Tavizón et al 2022). Given this situation, it has been proven in ewes and goats that appetitive sexual behavior is important to stimulate the reproductive female without the use of synthetic hormones (Abecia et al 2020), a favorable situation for animal reproduction since the concept is implemented “clean, green and ethical” or, a phenomenon known as the male effect commonly used for the reproduction of small ruminants (Martin and Kadokawa 2006).

On the other hand, consummatory sexual behavior ends with copulation by the female. The present study found that males had a higher copulation percentage on the third

day of contact between the sexes than TG-1 and TG-2. Consistent with this sexual behavior, it was reported in seasonally anestric goats where the females were synchronized and showed signs of estrus; because of this, males preferred females with estrus behavior over those that did not show signs of estrus (Giriboni et al 2017; Abecia et al 2020; Grimaldo-Viesca et al 2020). The foregoing shows that the display of appetitive sexual behavior is necessary to stimulate the sexual response in females. On the other hand, the males placed in contact with females in estrus avoid the courtship process, copulating without the occurrence of appetitive sexual behavior.

4.3. Environmental conditions

In the present study, the THI was found to be up to 90 U in the afternoon, evidencing severe heat stress conditions in domestic animals (Marai et al 2007). For example, ewes are considered to begin to experience heat stress at 22.2 U (THI), (22.2 and < 23.3 U moderate stress; 23.3 and < 25.6 U severe stress and ≥ 25.6 U extreme heat stress) (Vicente-Pérez et al 2020; Barragán-Sierra et al 2021). In the present study, the combination of temperature and humidity was high, predisposing rams to present problems in sexual behavior and seminal characteristics. However, according to the results, reproductive behavior in ewes and sexual behavior in rams were not affected by environmental conditions. This is attributed to the fact that the females did not have problems expressing sexual behavior due to the breed's adaptation to the tropical weather. Likewise, the sexual behavior of the males was not affected because the measurements were made in the cool morning hours. However, adverse effects on sexual behavior and seminal characteristics of rams have been observed under heat stress conditions (Hamilton et al 2016; Barragán-Sierra et al 2021). It is necessary to carry out more research regarding the effect of heat stress in the tropics and how it affects sexual performance at different times of the day, as well as the impact on the sperm quality of these rams.

5. Conclusions

Ewes treated with eCG showed a good reproductive behavior, and most did so in the first 24 to 36 h after removal of the intravaginal device. On the other hand, it was found that the rams in the first two days of contact showed intense appetitive sexual behavior. On the third day, when most females were in estrus, there was more consummatory sexual behavior. Finally, the environmental conditions of the tropics of Guerrero based on the THI are indicative of heat stress in hair ewes, which represents an extra challenge for the production and reproduction of sheep. This opens new research perspectives regarding ovarian dynamics and sexual behavior.

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Ethical considerations

All procedures and conditions of use, care, and welfare of the experimental ewes were carried out following national and international standards (NAM 2010; FASS 2010).

Conflict of interest

The authors declare no competing interests.

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