UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

“Los machos cabríos fotoestimulados mantienen elevada la LH en las hembras caprinas durante el anestro estacional, y los machos cabríos familiares (conocidos) inducen la ovulación en las cabras anéstricas”

TESIS

Que presenta ALFONSO LONGINOS MUÑOZ BENÍTEZ

como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS AGRARIAS

Torreón, Coahuila Diciembre de 2016

Los machos cabríos fotoestimulados mantienen elevada la LH en las hembras caprinas durante el anestro estacional, y los machos cabríos familiares (conocidos) inducen la ovulación en las cabras anéstricas

TESIS

Elaborada por **ALFONSO LONGINOS MUÑOZ BENÍTEZ** como requisito parcial para obtener el grado de Doctor en Ciencias Agrarias con la supervisión y aprobación del Comité de Asesoría

Dr. José Alberto Delgadillo Sánchez
Asesor Principal

Dr. Gerardo Duarte Moreno
Asesor

Dr. Matthieu Keller
Asesor

Dr. Luis Ángel Zarazaga Garcés
Asesor

Dr. José Alfredo Flores Cabrera
Asesor

Dr. Horacio Hernández Hernández
Asesor

Dr. Jesús Vielma Sifuentes
Asesor

Dr. Alberto Sandoval Rangel
Subdirector de Postgrado

Dr. Gonzalo Fitz Rodríguez
Jefe del Departamento de Postgrado

Torreón, Coahuila — Diciembre de 2016

Agradecimientos

A Dios, por permitirme vivir para alcanzar las metas que me he planteado y por todas las bendiciones que me da.

A mi asesor principal, el Dr. José Alberto Delgadillo por todas las enseñanzas a lo largo de mi postgrado. Gracias por su paciencia y por su dedicación al transmitir sus conocimientos.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca que se me otorgó para mis estudios de Doctorado (CVU-372022).

A los programas CONACYT-Ciencia Básica (254176): “Control de la reproducción caprina por las interacciones socio-sexuales”; CONACYT (México)- ANR (Francia; 159884; 11-ISV7-001-01): “El efecto macho en ungulados: reproducción aplicada y mecanismos neuroendocrinos”; SEP-CONACYT-ANUIES (México)-ECOS (Francia; M13A01): “Regulación de la estacionalidad de la reproducción por las interacciones sociales y el fotoperiodo en los caprinos”, por el apoyo financiero que me otorgaron durante mis estudios.

A mi comité particular de asesoría: Dr. Gerardo Duarte Moreno, Dr. Matthieu Keller, Dr. Luis Angel Zarazaga Garcés, Dr. José Alfredo Flores Cabrera, Dr. Horacio Hernández Hernández, Dr. Jesús Vielma Sifuentes, por todas las aportaciones que tuvieron para mi formación, y sobre todo por la amistad que forjamos durante mi estancia en el postgrado.

A la Dra. Ilda Graciela Fernández García y al Dr. Gonzalo Fitz Rodríguez por su valiosa aportación en los trabajos experimentales, y los consejos que me brindaron durante todo este tiempo.

A la Dra. Marie Bedos por su amistad, y por todas sus contribuciones que amablemente me brindó en los trabajos de campo, y en la preparación de mis publicaciones.

A Didier Chesneau, Anne-Lyse Lainé y a todos los miembros de la plataforma de determinaciones hormonales del Laboratorio de Fisiología de la Reproducción y del Comportamiento del INRA de Nouzilly, Francia, por realizar las determinaciones hormonales de mis trabajos experimentales.

A todos y cada uno de mis compañeros y amigos, por brindarme su apoyo y por poner todo su empeño al ayudarme en mis trabajos experimentales.

Al Sr. Enrique Urquízo Sánchez, Ing. Enrique Antequera, y a la M.V.Z. Karla Ríos, por facilitarme sus animales para la realización de mis estudios experimentales.

A los señores Jesús García y Julio Zalazar por el cuidado y alimentación de los animales experimentales en la Universidad Autónoma Agraria Antonio Narro, Unidad Laguna.

A la familia Díaz Herrera por todo su apoyo y cariño que me brindan para poder seguir adelante.

A la familia Martínez Benítez por todo el cariño y el ejemplo de lucha que me han dado a lo largo de mi vida.

Dedicatoria

Para Ana Lucía, motor de mi lucha para ser mejor cada día, y por permitirme ser un ejemplo y guía en su vida. Te amo “Chila”.

Para Laura, fuente inagotable de amor y apoyo; gracias por ser mi amiga y mi fortaleza en los momentos buenos, pero sobre todo en los momentos difíciles. Te amo “Lau”.

Para mi madre, la Sra. Quirina Benítez Guerrero, por ser mi mayor ejemplo de lucha por la vida, y por haber sido siempre mi mejor amiga. Gracias por no dejar que me rindiera, por tantos consejos y momentos felices. Te extraño y te amo por siempre. *“Llegará el día en que pueda verte sin cerrar los ojos”*.

Para mi padre Longinos Muñoz Vázquez, por ser mi ejemplo para seguir siempre hacia adelante, y de lucha incansable en la vida. Te amo “Pa”.

Para mis hermanos Luis y Ana Muñoz Benítez, por ser mis amigos, compañeros y mi apoyo incondicional, los amo.

Para el Sr. Jesús Martínez Huerta y la Sra. Magdalena Benítez Guerrero, por ser mis segundos padres y ejemplo para ser mejor cada día. Gracias eternas por su amor y apoyo.

Physiology & Behavior 158 (2016) 137–142

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Efficiency of the male effect with photostimulated bucks does not depend on their familiarity with goats

A.L. Muñoz [a], M. Bedos [a], R.M. Aroña [a], J.A. Flores [a], H. Hernández [a], C. Moussu [b], E.F. Briefer [c], P. Chemineau [b], M. Keller [b], J.A. Delgadillo [a,*]

[a] Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez y Carretera a Santa Fe, C.P. 27054 Torreón, Coahuila, Mexico
[b] Physiologie de la Reproduction et des Comportements, INRA, CNRS, Université de Tours, IFCE, Agreenium, Nouzilly, France
[c] ETH Zürich, Institute of Agricultural Sciences, Universitätstrasse 2, 8092 Zürich, Switzerland

HIGHLIGHTS

- We compared the sexual response of female goats exposed to familiar or novel bucks.
- These males were either sexually active or sexually inactive.
- Sexual active males stimulated sexual response in more goats than sexually inactive.
- Familiarity of sexually active males did not decrease the sexual response of goats.
- Novelty of sexually inactive males did not stimulate the sexual activity of goats.

ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 19 February 2016
Accepted 26 February 2016
Available online 3 March 2016

Keywords:
Social recognition
Familiarity
Male effect
Anovulatory goats
Ovulation
Photoperiodic treatment

ABSTRACT

In ewes, the ovulatory response of females exposed to familiar rams is lower than the response of those exposed to novel ones. In goats, males rendered sexually active by exposure to long days are more efficient to induce ovulation in seasonal anestrous females than untreated males. Two experiments were conducted to determine 1) whether male goats remain familiar to females after 45 days of separation; and 2) whether photostimulated males are able to stimulate the sexual activity of females, independently of their familiarity with them. In Experiment 1, three groups of goats (n = 10 goats per group) were put in contact with males (n = 2 per group) during 10 days in November (familiarization period). These males were called familiar males. After 15, 30 and 45 days of separation from the males, females of each group were exposed to familiar or novel males during 10 min. In each test, goats in contact with novel males displayed more distress bleats, escapes, head butts, and sniffing than those in contact with familiar males ($P < 0.05$). In Experiment 2, we used sexually inactive (n = 4 control males), and sexually active males (n = 4 photostimulated males). In February, two groups of goats (n = 50 each) were put in contact with control or photostimulated males (n = 2 each) during 10 days ("familiar" control or photostimulated male, respectively). After 45 days of separation from the males, both groups of females were further divided into two groups (n = 25 goats per group). In April, two groups were re-exposed to "familiar" control or "familiar" photostimulated males (n = 2 per group), whereas the other two groups were exposed to "novel" control or "novel" photostimulated males (n = 2 per group). The photostimulated males displayed a higher level of sexual behavior than the controls. The proportion of goats that ovulated and displayed estrus was higher when exposed to the photostimulated males than when exposed to control ones (≥80% vs. 0%; $P < 0.05$). These proportions did not differ between groups exposed to familiar or novel photostimulated males ($P > 0.05$). We concluded that after 45 days of separation, males are still familiar to females. The photostimulated males are able to induce the sexual activity of seasonally anestrous goats independently of their familiarity with them.

1. Introduction

In breeds of goats and ewes that display reproductive seasonality, the introduction of a male into a group of seasonal anovulatory females induces an immediate increase in the secretion of LH, leading to

* Corresponding author at: Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez y Carretera a Santa Fe, 27054 Torreón, Coahuila, Mexico.
E-mail address: joaldesa@yahoo.com (J.A. Delgadillo).

http://dx.doi.org/10.1016/j.physbeh.2016.02.037
0031-9384/

ovulation associated or not with estrous behavior. This phenomenon is known as the "male effect" [1–3]. Many factors influence the endocrine and ovulatory responses of females exposed to males, among which are the intensity of the male's sexual behavior, the degree of familiarity with males, and the previous period of separation between sexes.

In small ruminant females showing a strong seasonality, the endocrine and sexual changes associated with the male effect are low or absent in most females during the mid-anestrus, probably due to a decrease of the sexual behavior of males that are also in sexual rest [4–6]. However, when bucks are submitted to artificial long days followed by natural photoperiod to stimulate their sexual behavior during the natural sexual rest, these males become able to stimulate the secretion of LH, estrous behavior and ovulation in most anestrous females [7–9]. These results suggest that the intensity of sexual behavior of males is an important factor for the success of the male effect.

Another factor that could modify the effectiveness of the male effect is the use of "familiar males", i.e. males already known by females. Indeed, different studies suggest that familiarity plays an important role in social recognition. In fact, it was demonstrated that goats are able to recognize group members relying on olfactory and visual cues [10]. Interestingly, in sheep, females are able to remember familiar individuals over very long periods of time (>2 months; [11]). In addition, Keller et al. [12] demonstrated that ewes were able to recognize familiar flock partners in comparison to a completely unfamiliar female after only 24 h of direct contact. More interestingly, it was shown that the endocrine response of females is higher when they are exposed to novel males in comparison to familiar ones. Indeed, when ewes were exposed for 3 months to males (familiar males), and re-exposed to familiar or novel males after 15 min of separation during the anestrus season, only the novel males were able to increase the frequency of LH pulses [13]. In another study, females remained with males during 3 months, and were then re-exposed to them (familiar males) or exposed to novel males after 15 min or 1 month of separation [14]. This study demonstrated that novel rams increase the pulsatile LH secretion and stimulate LH surge in all females after either 15 min or 1 month of separation, whereas familiar ones are able to increase pulsatile LH secretion only after 1 month of separation and stimulate a preovulatory LH surge in only a third of females [14]. Overall, these results suggest that in ewes, the familiarity of males plays an important role in the response of females to males. In addition, these results suggest that familiar males may become novel again after about 1 month of separation of females.

For decades, it has been thought that females must be previously isolated from males to respond to the male effect. In ewes, it was shown that periods of 21 or 17 days of isolation were sufficient to ensure an ovulatory response in females exposed to males [15,16]. Furthermore, Cohen-Tannoudji and Signoret [17] found that ewes showed an increase of LH pulsatility when they were re-exposed to males after 24 h of separation. Similarly, in goats, it was suggested that a period of at least 3 weeks of separation between sexes was necessary so that females were able to display the male effect [2]. However, it was demonstrated that previous separation between sexes is not necessary when males are naturally sexually active or stimulated by exposure to artificially long days [18,19]. In fact, in one of these studies, most females (9/11) that remained during about three months with sexually inactive males due to the seasonal quiescence, displayed estrous behavior and ovulated when they were re-exposed to photostimulated bucks. In contrast, no females displayed estrous behavior and only 1/11 female ovulated when they were re-exposed to another sexually inactive buck [19]. These later results suggest that the sexual behavior of males plays an important role in inducing the sexual activity of does. However, it is difficult to interpret the results reported by Véliz et al. [19], because the study does not mention if the sexually active or inactive males that were re-introduced with females were familiar or novel bucks.

Considering that photostimulated bucks are very efficient in inducing the sexual activity of seasonally anestrous females, we hypothesized that sexually active bucks would be able to stimulate the estrus and ovulatory activities of females, independently of their familiarity with them. To test this possibility, we exposed females in seasonal anestrus to a 10-days period of "familiarization" with males that were either subjected to a photoperiodic treatment or kept under natural photoperiod, and re-exposed them to either a familiar or novel male after 45 days of separation.

2. Materials and methods

2.1. General

The procedures used in this experiment were in strict accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals [20].

This study was conducted during the non-breeding season using local goats from Laguna Region at the State of Coahuila, Mexico (latitude 26° 23′ N, longitude 104° 47′ W). The photoperiod in this region varies from 13 h 41 min of light at the summer solstice to 10 h 19 min of light at the winter solstice. The seasonal anestrus in females isolated from males occurs from February-March to August-September; the sexual rest in bucks isolated from females occurs from December-January to May-June [21,22]. During the whole study, females and males were fed with 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate feed (14% CP; 1.7 Mcal/kg) with free access to water and mineral blocks.

2.2. Experiment 1

2.2.1. Females and familiarization period

On November 15th, female goats were divided in three groups ($n = 10$ each), and kept in three, 10×10 m, different shaded open pens. The distance between pens was at least 100 m. On November 20th, the females of each group were put in direct physical contact with males ($n = 2$) and remained with them during 10 days. The aim of this procedure was that males become "familiar" to females. After the 10-days period of contact, on November 30th, males were removed from the three groups of females and put in another pen located at least 200 m from the pens of females. Females of each group remained together until the recognition tests were performed.

2.2.2. Measurements

With the aim to prove that females are able to recognize males after a previous separation period of 15, 30 and 45 days, we used the paired closed encounters test, which has been previously used to test the discrimination between familiar or novel individuals in sheep [23,12]. Each doe was individually tested in two stages: for half of the females, the test was performed first with a familiar male and second with a novel male. By contrast, the other half of the females was tested first with a novel male and second with a familiar male. The time between the first test and the second test was around 2 h. Before starting the test, each doe was removed from its pen and introduced into an independent pen test (2×2 m) during 2 min. After this period, one "familiar" or "novel" male (no prior contact with females) was introduced during 10 min and the following behaviors displayed by the doe were scored; the number of distress bleats, escapes, head butts, and sniffing. After the tests, each doe was placed in a new independent pen with the aim of avoiding the transmission of smell of the male to the other females.

2.2.3. Statistical analyses

The Wilcoxon Signed-Rank test was used to compare the behaviors displayed by each female in presence of familiar or novel males within each test. The Kruskal-Wallis test was used to compare the behaviors displayed by females in presence of familiar or novel males between each test.

2.3. Experiment 2

2.3.1. Stimulation of sexual behavior of males

We used eight adult bucks that had never been in contact with the females used in this experiment (see Section 2.3.3). Four bucks were kept under natural variations of day-length during the whole experiment. These males were the control males. Another four adult male goats were subjected in a 10 m × 5 m shaded open pen to a photoperiodic treatment to induce their sexual activity during the non-breeding season as described previously [24]. These bucks were the photostimulated males. Briefly, males were exposed to artificial long days (16 h of light/8 h of darkness) from November 1st to January 15th. From January 16th on, males were exposed to natural variations of day-length until the end of the study. This photoperiodic treatment stimulates testosterone secretion in March and April and, as a consequence, improves the intensity of sexual behavior and odor during the months corresponding to the non-breeding season [24,25].

2.3.2. Females and familiarization period

On January 27th, non-pregnant female goats were divided in two groups balanced for body condition score (BCS; [26]) and kept in two different, 10 × 10 m, shaded open pens. On February 3rd, one group of does (n = 50; BCS: 2.0 ± 0.2; mean ± SEM) was put in direct physical contact with the control males (n = 2), whereas another group of does (n = 50; BCS: 2.1 ± 0.1) was put in direct physical contact with the photostimulated males (n = 2). The distance between these pens was about 100 m. The photostimulated males were put in contact with the does 19 days after the end of the photoperiodic treatment, when they were still sexually quiescent, and the control males were already in sexual rest [24]. The contact between females and males lasted 10 days, in order to expose both groups of females to sexually inactive males. On February 13th, control and photostimulated males were removed from both groups of does. Females from both groups remained together in their respective open pens until the males were re-introduced (see below). The objective of this pre-exposure period was that control and photostimulated males became "familiar" to does [11,12]. Thereafter, the males used during the familiarization period were called familiar control or familiar photostimulated males.

2.3.3. The male effect

On March 14th, 21st and 28th, all the multiparous goats (n = 100) exposed in February to control or photostimulated males were submitted to a transrectal ultrasonography to determine their ovarian cyclicity using an Aloka SSD-500 scanner connected to a transrectal 7.5 MHz linear probe. None of the females presented corpus luteum. Therefore, all of them were considered in seasonal anovulation. On March 28th, the two groups of goats were further divided (n = 25 each) according to their BCS: goats exposed in February to control males were divided in two groups. Thereafter, on April 2nd (day 0 at 08:00), one group (BCS: 1.9 ± 0.1) was re-exposed to the "familiar" control males (n = 2), whereas the other one (BCS: 1.9 ± 0.3) was exposed to the "novel" control males (n = 2). Females previously exposed to the photostimulated males were also divided in two groups: one group (BCS: 1.9 ± 0.3) was re-exposed to the "familiar" photostimulated males (n = 2), whereas the other one (BCS: 1.9 ± 0.1) was exposed to the "novel" photostimulated males (n = 2). Each group of females exposed to males was divided into two sub-groups so that each buck individually stimulated 12 or 13 females. The four groups of females remained in contact with their respective males for 18 days. The distance between the four groups of females was about 100 m to prevent any visual or auditory contact between groups.

2.3.4. Measurements

To ensure that bucks used in this experiment displayed levels of sexual behavior corresponding to their respective group, sexual behavior was individually recorded for 1 h by trained observers, from 08:00 to 09:00, on day 0 and 1 following their introduction into the groups of females. One hour of observation in the first two days after male introduction allowed to discriminate the differences in sexual behavior between the sexually active and inactive male goats [27,28]. The following behaviors were recorded; nudging, ano-genital sniffing, mount intention movements, self-urination and flehmen [5,29,30].

The ovarian activity was assessed by the presence of corpora lutea observed in each female by transrectal ultrasonography 6 and 19 days after the introduction of males using the same equipment (see Section 2.3.3). In addition, the presence of corpora lutea was confirmed by progesterone levels. For this aim, blood samples were obtained daily from each female from day 0 to day 9 and every 3 days from day 12 to day 18 after the introduction of males. All 5-mL samples were collected by jugular venipuncture in tubes containing 30 μL of heparin and centrifuged immediately at 3500 × g for 30 min; the obtained plasma was stored at − 20 °C until progesterone concentrations were measured by immunoenzymatic assay as described by Canépa et al. [31]. Sensitivity was 0.25 ng/mL. The intra- and inter-assay coefficients of variation were 8 and 10%, respectively. Females in which progesterone increased to ≥ 1.0 ng/mL were considered to have ovulated [32].

Estrous behavior of females was monitored twice daily (between 08:00 and 09:00 and between 18:00 and 19:00) during the 18 days of the study. A female was considered in estrus if she stood immobile when mounted by the male [33]. A high percentage of female goats displayed short estrous cycles when exposed to the sexually active males. Therefore, in this study, the percentage of these cycles and their duration were inferred from the monitoring of estrus, and duration was defined as the number of days between the beginnings of two consecutive estruses. Duration of short estrous cycle is b17 days [33]. In females that displayed short estrous cycles, the estrus-ovulation association was determined at first and second ovulation, whereas in females that displayed normal estrous cycles, this association was determined at the first ovulation. In both cases, results were reported as the percentage of females that displayed estrus accompanied by ovulation.

2.3.5. Statistical analyses

The percentages of females that showed estrous and/or ovulation, short or normal ovulatory cycles and the association between estrous

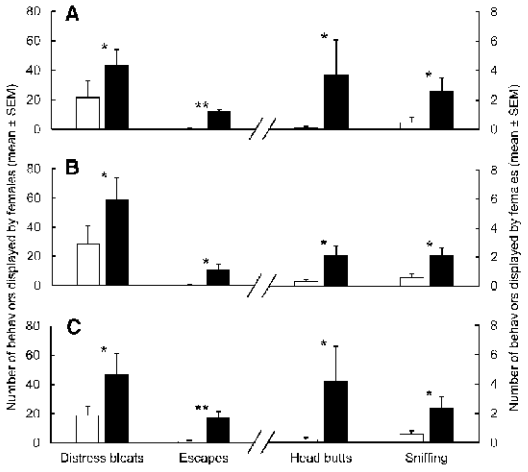

Fig. 1. Number of behaviors (mean ± SEM) displayed by female goats individually exposed during 10 min to "novel" (■) or "familiar" (□) bucks after 45 days (A), 30 days (B) or 15 days (C) of separation. Prior to the tests, females of each group remained in contact with males (n = 2) during 10 days (familiarization period). These were called the "familiar males" (*P b 0.05).

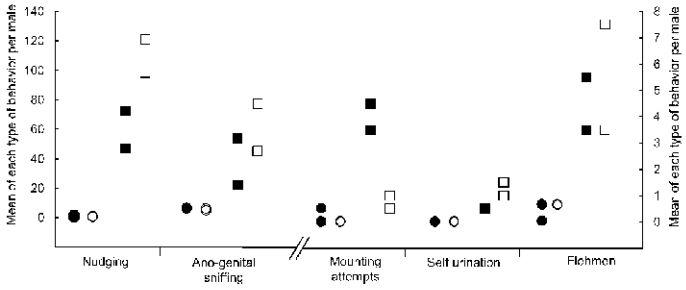

Fig. 2. Sexual behavior of males. Individual values of mean occurrences of nudging, ano-genital sniffing, mounting attempts, self-urination and flehmen in males (n = 2 per group) interacting with seasonal anestrous goats. Familiar (●) or novel (○) control, sexually inactive males, were kept under natural variations of day-length during the whole experiment. Familiar (■) or novel (□) photostimulated males were rendered sexually active by exposure to artificial long days (16 h of light per day) from November 1st to January 15th followed by natural photoperiodic conditions. Sexual behavior of males was observed from 08:00 to 09:00 on day 0 and 1 following their introduction in the groups of females.

and ovulation were compared by Chi-Square test. A two-sample t-test was used to assess statistical differences between the duration of short ovulatory cycles. Sexual behavior of bucks could not be statistically analyzed due to the low number of males in each group (n = 2).

Analyses were computed using the statistical package SYSTAT 13 [34]. Data were expressed as the mean ± standard error of the mean and differences were considered significant at the level of $P \leq 0.05$.

3. Results

3.1. Experiment 1

3.1.1. Behavior of females

Goats in contact with novel males displayed more distress bleats, escapes, head butts, and sniffing at 15, 30, and 45 days than those in contact with familiar males ($P < 0.05$; Fig. 1). However, time did not affect the evolution of these variables between successive expositions ($P > 0.05$), suggesting a strong and long-term maintenance of this social recognition.

3.2. Experiment 2

3.2.1. Sexual behavior of males

Sexually active males seemed to display a higher level of sexual behavior than sexually inactive ones. Indeed, the occurrences of all the behaviors considered, including nudging, ano-genital sniffing, mounting attempts, self-urination and flehmen expressed by the photostimulated males were higher than those expressed by control ones (Fig. 2).

3.2.2. Estrous and ovulatory responses of goats

The proportion of goats that ovulated or showed estrous behavior at least once was higher in the groups exposed to familiar or novel photostimulated males, than in the groups exposed to familiar or novel control males ($P < 0.05$; Table 1; Figs. 3 and 4). Indeed, during the whole study, none of the goats in contact with familiar or novel control males displayed estrous behavior or ovulation. In contrast, most goats exposed to familiar or novel photostimulated males showed estrous behavior (≥80%) and ovulated (96%) at least once during the whole study. The proportion of females that showed estrous behavior or ovulated did not differ among groups exposed to familiar or novel photostimulated males ($P > 0.05$). In addition, the proportion of goats that displayed normal or short estrous cycles did not differ between groups ($P > 0.05$). Finally, in females exposed to familiar or novel photostimulated males and displaying short estrous cycles, the estrus-ovulation association at the first (75% vs. 70%) and second (100% vs. 100%) ovulation did not differ between groups ($P > 0.05$). Similarly, in females exposed to familiar or novel photostimulated males displaying normal estrous cycles, the estrus-ovulation association (67% vs. 100%) did not differ between groups ($P > 0.05$). After day 10, no estrous behavior or ovulations were recorded (Fig. 3).

4. Discussion

The aim of this study was to determine whether males remain familiar to females after 45 days of separation, and to whether photostimulated males are able to stimulate the sexual activity of females, independently of their familiarity with them. Therefore, in this study we showed that males remain familiar to females after 45 days of separation, and that photostimulated bucks are able to induce sexual activity in seasonally anestrous goats, independently of whether they are "familiar" or "novel" males. Indeed, the percentages of females that displayed estrous behavior or ovulated when exposed to photostimulated males was high and did not differ between does exposed to the familiar or novel photostimulated males. In addition, the characteristics of the sexual responses to the introduction of males, such as the percentages of short or normal estrous cycles, and the duration of the short cycles did not differ between females in contact with the familiar or novel photostimulated males. In contrast, neither estrous behavior nor ovulations were observed in goats exposed

Table 1
Estrus and ovulatory responses of anestrous female goats exposed to familiar or novel control, sexually inactive males that were kept under natural variations of day-length, or exposed to familiar or novel photostimulated males rendered sexually active by exposure to long days (16 h of light per day) from November 1st to January 15th followed by natural photoperiodic conditions.

Groups of females in contact with:	n	Females with ovulations (%)	Females with estrus (%)	Females with normal estrous cycles (%)	Females with short estrous cycles (%)	Duration of short estrous cycles (days)□
Familiar control males	25	0[a]	0[a]	–	–	–
Novel control males	25	0[a]	0[a]	–	–	–
Familiar photostimulated males	25	96[b]	80[b]	27[b]	73[b]	5.9 ± 0.2[b]
Novel photostimulated males	25	96[b]	88[b]	23[b]	77[b]	5.4 ± 0.3[b]

[ab]Values with different letters within each column are different ($P < 0.05$).
□ The latency to first estrus and the duration of short ovarian cycles were expressed as the mean ± standard error of the mean.

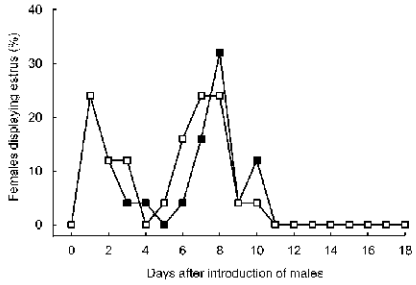

Fig. 3. Estral response of goats exposed to males. Daily percentages of females that displayed estrous behavior after introduction of familiar (■; n = 25) or novel (□; n = 25) photostimulated males. None of the females exposed to familiar or novel control males showed estrous behavior and are therefore not represented in the figure. The photostimulated males were rendered sexually active by exposure to artificial long days (16 h of light per day) from November 1st to January 15th followed by natural photoperiodic conditions.

to the familiar or novel control males. Our results are in accordance with previous studies that showed that photostimulated males are able to induce sexual activity in most females, whereas the control bucks are unable to do so in seasonally anestrous goats [5,35,36]. Taken together, these findings confirm our hypothesis that sexually active males are able to stimulate the sexual activity of female goats, independently of their familiarity with them. In addition, our results support the hypothesis

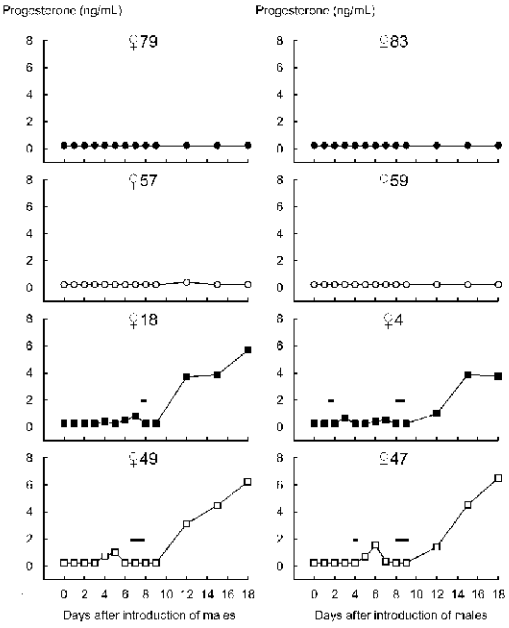

Fig. 4. Representative profiles of plasma concentration of progesterone of goats exposed to males. Individual patterns of plasma progesterone concentration in goats exposed to familiar (●) or novel (○) control, sexually inactive males, or to familiar (■) or novel (□) photostimulated males. The photostimulated males were rendered sexually active by exposure to artificial long days (16 h of light per day) from November 1st to January 15th followed by natural photoperiodic conditions. The bars indicate the duration of estrous behavior.

that in goats, the sexual behavior of males is a key factor to stimulate the estrous and ovulatory activities of females subjected to the male effect.

Our results showed that the exposition to novel males did not increase the sexual response of females, unless these males were sexually active. Indeed, most females displayed estrous behavior and ovulated when exposed to the photostimulated males, independently of whether they were familiar or novel. In contrast, no females displayed sexual activity when in contact with control males, independently of whether they were familiar or novel. Our results are not consistent with those reported in sheep, in which novelty of male stimulus is considered to be an important factor to stimulate the endocrine and sexual activities in ewes [13,14]. In fact, all ewes displayed a LH surge when they were exposed to novel males after 1 month of separation, whereas familiar males were able to induce a LH surge only in a third of females after the same period of separation [14]. Based on these later results, it was suggested that familiar rams might have become unfamiliar again after one month of separation from females [14]. However, this hypothesis was not validated here in goats, and seems in contradiction with results indicating that ewes are able to remember familiar individuals over very long periods of time (N2 months; [11]). In the present study, even if males and females remained separated for 45 days after the familiarization period, control novel males did not trigger a better sexual response in females than familiar ones. The reasons for the difference between our results and those reported in sheep are not clear, but this difference could be related to species, breeds or to the fact that we used photostimulated males, which displayed an intense sexual activity [3,6,37].

The importance of male sexual behavior in triggering endocrine and ovulatory responses in seasonally anovulatory female goats from subtropical regions has been well described in the literature. Indeed, this postulate is supported by several studies published by our group. Firstly, Delgadillo et al. [36] showed that females that remain with control males start their ovulatory activity in June, when males become sexually active. When females were exposed to photostimulated males, they started to ovulate before the onset of the natural breeding season, when males increased their sexual behavior. Secondly, it was shown that goats that remained in contact with males during three months ovulate when they are re-exposed to photostimulated, sexually active bucks, but not when they are re-exposed to control, sexually inactive ones [19]. Thirdly, in another study using goats of the same breed as used in the current study, none of them experienced a LH surge nor ovulation when exposed to sedated, photostimulated males, which emitted a strong odor but did not display any sexual behavior. In contrast, does exposed to intact, photostimulated males, which emitted a strong odor and displayed an intense sexual behavior, did so [38]. In the present study, the sexual behavior of the photostimulated males was more intense than that displayed by the control ones. Therefore, we can suppose that the photostimulated males were able to reactivate GnRH/LH secretion, thus allowing ovulation; whereas the control males did not do so. Interestingly, the reactivation of the gonadotropic axis induced by the photostimulated males was independent of whether they were familiar or novel. Altogether, our results and those mentioned above indicate that, in goats, the sexual behavior displayed by males is more important than the familiarity between males and females in inducing the ovulatory activity in seasonally anestrous goats exposed to the male effect.

Acknowledgments

The authors are grateful to Enrique Antequera and Karla Ríos from Almerimex company for providing the female goats used in the Experiment 1; to Enrique Urquízo for providing the female goats used in the Experiment 2; and to Julio Salazar and Jesús García for taking care of the animals at the Centro de Investigación en Reproducción Caprina (CIRCA). We also thank Anne-Lyse Lainé and all the members of the platform of hormonal assay of the Laboratoire de Physiologie de

la Reproduction et des Comportements of the INRA of Nouzilly, France, for carrying out the progesterone assay. We also express our thanks to all members of the CIRCA of the Universidad Autónoma Agraria Antonio Narro for their technical assistance, particularly to Jesús Vielma, Gerardo Duarte and Gonzalo Fitz-Rodríguez. We are grateful to Dolores López for their efficient secretarial and administrative assistance. A. L. Muñoz was supported by a scholarship of CONACYT during his doctoral studies (number of scholarship CVU-372022). This work was supported by grants from the "Programa de Cooperación CONACYT" (México) - Agence Nationale de la Recherche (France) 159884: "El efecto macho en ungulados: reproducción aplicada y mecanismos neuroendocrinos/ ANR Blanche Internationale France-Mexique" (11-ISV7-001–01).

References

[1] G.B. Martin, C.M. Oldham, Y. Cognié, D.T. Pearce, The physiological response of anovulatory ewes to the introduction of rams. A review, Livest. Prod. Sci. 15 (1986) 219–247, http://dx.doi.org/10.1016/0301-6226(86)90031-X.
[2] P. Chemineau, Possibilities for using bucks to stimulate ovarian and oestrous cycles in anovulatory goats. A review, Livest. Prod. Sci. 17 (1987) 135–147, http://dx.doi.org/10.1016/0301-6226(87)90059–5.
[3] J.A. Delgadillo, H. Gelez, R. Ungerfeld, P.A.R. Hawken, G.B. Martin, The 'male effect' in sheep and goats-revisiting the dogmas, Behav. Brain Res. 200 (2009) 304–314 http://dx.doi.org/10.1016/j.bbr.2009.02.004.
[4] B.J. Restall, Seasonal variation in reproductive activity in Australian goats, Anim. Reprod. Sci. 27 (1992) 305–318, http://dx.doi.org/10.1016/0378-4320(92)90145–4.
[5] J.A. Flores, F.G. Véliz, J.A. Pérez-Villanueva, G. Martínez de la Escalera, P. Chemineau, P. Poindron, et al., Male reproductive condition is the limiting factor of efficiency in the male effect during seasonal anestrus in female goats, Biol. Reprod. 62 (2000) 1409–1414http://dx.doi.org/10.1095/biolreprod62.5.1409.
[6] A. Chanvallon, L. Sagot, E. Pottier, N. Debus, D. Francois, T. Fassier, New insights into the influence of breed and time of the year on the response of ewes to the 'ram effect', Animal 5 (2011) 1594–1604 http://dx.doi.org/10.1017/S1751731111000668.
[7] J. Vielma, P. Chemineau, P. Poindron, B. Malpaux, J.A. Delgadillo, Male sexual behavior contributes to the maintenance of high LH pulsatility in anestrous female goats, Horm. Behav. 56 (2009) 444–449 http://dx.doi.org/10.1016/j.yhbeh.2009.07.015.
[8] M. Bedos, G. Duarte, J.A. Flores, G. Fitz-Rodríguez, H. Hernández, J. Vielma, et al., Two or 24 h of daily contact with sexually active males results in different profiles of LH secretion that both lead to ovulation in anestrous goats, Domest. Anim. Endocrinol. 48 (2014) 93–99 http://dx.doi.org/10.1016/j.domaniend.2014.02.003.
[9] L.A. Zarazaga, I. Celi, J.L. Guzmán, B. Malpaux, Enhancement of the male effect on reproductive performance in female mediterranean goats with long day and/or melatonin treatment, Vet. J. 192 (2012) 441–444 http://dx.doi.org/10.1016/j.tvjl.2011.09.012.
[10] N.M. Keil, S. Imfeld-Mueller, J. Aschwanden, B. Wechsler, Are head cues necessary for goats (*Capra hircus*) in recognising group members? Anim. Cogn. 15 (2012) 913–921http://dx.doi.org/10.1007/s10071-012-0518-6.
[11] K.M. Kendrick, A.P. Da Costa, A.E. Leigh, M.R. Hinton, J.W. Peirce, Sheep don't forget a face, Nature 414 (2001) 165–166.
[12] M. Keller, F. Cornilleau, E. Archer, F. Lévy, Development of social familiarity in ewes, Physiol. Behav. 104 (2011) 392–397 http://dx.doi.org/10.1016/j.physbeh.2011.04.030.
[13] P.A.R. Hawken, T. Jorre de St. Jorre, J. Rodger, T. Esmaili, D. Blache, G.B. Martin, Rapid induction of cell proliferation in the adult female ungulate brain (*Ovis aries*) associated with activation of the reproductive axis by exposure to unfamiliar males, Biol. Reprod. 80 (2009) 1146–1151 http://dx.doi.org/10.1095/biolreprod.108.075341.
[14] T. Jorre de St Jorre, P.A.R. Hawken, G.B. Martin, Role of male novelty and familiarity in male-induced LH secretion in female sheep, Reprod. Fertil. Dev. 24 (2012) 523–530 http://dx.doi.org/10.1071/RD11085.
[15] C.M. Oldham, Y. Cognie, Do ewes continue to cycle after teasing? Proc. Aust. Soc. Anim. Prod. 13 (1980) 82–85.
[16] C.M. Oldham, Stimulation of ovulation in seasonally or lactationally anovular ewes by rams, Proc. Aust. Soc. Anim. Prod. 13 (1980) 73–74.
[17] J. Cohen-Tannoudji, J.P. Signoret, Effect of short exposure to the ram on later reactivity of anoestrous ewes to the male effect, Anim. Reprod. Sci. 13 (1987) 263–268, http://dx.doi.org/10.1016/0378-4320(87)90063–7.
[18] L. Gallego-Calvo, M.C. Gatica, I. Celi, J.L. Guzmán, J.A. Delgadillo, L.A. Zarazaga, No previous isolation of female goats is required for novel males to induce a male effect, especially if direct physical contact is established, Theriogenology 82 (2014) 1310–1315 http://dx.doi.org/10.1016/j.theriogenology.2014.08.015.
[19] F.G. Véliz, P. Poindron, B. Malpaux, J.A. Delgadillo, Maintaining contact with bucks does not induce refractoriness to the male effect in seasonally anestrous female goats, Anim. Reprod. Sci. 92 (2006) 300–309 http://dx.doi.org/10.1016/j.anireprosci.2005.06.006.
[20] Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, NORMA Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio, Diario Oficial de la Federación, México DF, 22 August 2001, 2001.
[21] G. Duarte, J.A. Flores, B. Malpaux, J.A. Delgadillo, Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability, Domest. Anim. Endocrinol. 35 (2008) 362–370 http://dx.doi.org/10.1016/j.domaniend.2008.07.005.
[22] J.A. Delgadillo, G.A. Canedo, P. Chemineau, D. Guillaume, B. Malpaux, Evidence for an annual reproductive rhythm independent of food availability in male creole goats in subtropical northern Mexico, Theriogenology 52 (1999) 727–737, http://dx.doi.org/10.1016/S0093-691X(99)00166–1.
[23] R.H. Porter, L. Désiré, R. Bon, P. Orgeur, The role of familiarity in the development of social recognition by lambs, Behaviour 138 (2001) 205–219 http://dx.doi.org/10.1163/15685390151074384.
[24] J.A. Delgadillo, J.A. Flores, F.G. Véliz, H.F. Hernández, G. Duarte, J. Vielma, et al., Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days, J. Anim. Sci. 80 (2002) 2780–2786.
[25] R. Rivas-Muñoz, G. Fitz-Rodríguez, P. Poindron, B. Malpaux, J.A. Delgadillo, Stimulation of estrous behavior in grazing female goats by continuous or discontinuous exposure to males, J. Anim. Sci. 85 (2007) 1257–1263 http://dx.doi.org/10.2527/jas.2006-115.
[26] S.W. Walkden-Brown, B.J. Restall, R.J. Scaramuzzi, G.B. Martin, M.A. Blackberry, Seasonality in male Australian cashmere goats: long term effects of castration and testosterone or oestradiol treatment on changes in LH, FSH and prolactin concentrations, and body growth, Small Rumin. Res. 26 (1997) 239–252 http://dx.doi.org/10.1016/S0921-4488(97)00017-5.
[27] J. Vielma, P. Chemineau, P. Poindron, B. Malpaux, J.A. Delgadillo, Male sexual behavior contributes to the maintenance of high LH pulsatility in anestrous female goats, Horm. Behav. 56 (2009) 444–449 http://dx.doi.org/10.1016/j.yhbeh.2009.07.015.
[28] J.L. Ponce, H. Velázquez, G. Duarte, M. Bedos, H. Hernández, M. Keller, et al., Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of male goats to stimulate ovulatory activity in seasonally anovulatory females, Domest. Anim. Endocrinol. 48 (2014) 119–125 http://dx.doi.org/10.1016/j.domaniend.2014.03.002.
[29] R. Gonzalez, P. Poindron, J.P. Signoret, Temporal variation in LH and testosterone responses of rams after the introduction of oestrous females during the breeding season, J. Reprod. Fertil. 83 (1988) 201–208 http://dx.doi.org/10.1530/jrf.0.0830201.
[30] C. Fabre-Nys, Le comportement sexuel des caprins: contrôle hormonal et facteurs sociaux, INRA Prod. Anim. 13 (2000) 11–23.
[31] S. Canépa, A.L. Lainé, A. Bluteau, C. Fagu, C. Flon, D. Monniaux, Validation d'une méthode immunoenzymatique pour le dosage de la progestérone dans le plasma des ovins et des bovins, Cah Tech INRA, 64 2008, pp. 19–30.
[32] J.A. Delgadillo, J.A. Flores, O. Villarreal, M.J. Flores, G. Hoyos, P. Chemineau, et al., Length of postpartum anestrus in goats in subtropical Mexico: effect of season of parturition and duration of nursing, Theriogenology 49 (1998) 1209–1218 http://dx.doi.org/10.1016/S0093-691X(98)00068-5.
[33] P. Chemineau, A. Daveau, F. Maurice, J.A. Delgadillo, Seasonality of estrus and ovulation is not modified by subjecting female alpine goats to a tropical photoperiod, Small Rumin. Res. 8 (1992) 299–312 http://dx.doi.org/10.1016/0921-4488(92)90211-L.
[34] SYSTAT 13, 2009. Cranes Software International Ltd, San José, CA, USA
[35] J.L. Ponce, H. Velázquez, G. Duarte, M. Bedos, H. Hernández, M. Keller, et al., Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of male goats to stimulate ovulatory activity in seasonally anovulatory females, Domest. Anim. Endocrinol. 48 (2014) 119–125 http://dx.doi.org/10.1016/j.domaniend.2014.03.002.
[36] J.A. Delgadillo, J.A. Flores, H. Hernández, P. Poindron, M. Keller, G. Fitz-Rodríguez, et al., Sexually active males prevent the display of seasonal anestrus in female goats, Horm. Behav. 69 (2015) 8–15 http://dx.doi.org/10.1016/j.yhbeh.2014.12.001.
[37] J.A. Delgadillo, J.A. Flores, G. Duarte, J. Vielma, H. Hernández, M. Bedos, et al., Out-of-season control of reproduction in subtropical goats without exogenous hormonal treatments, Small Rumin. Res. 121 (2014) 7–11 http://dx.doi.org/10.1016/j.smallrumres.2014.01.011.
[38] J.C. Martínez-Alfaro, H. Hernández, J.A. Flores, G. Duarte, G. Fitz-Rodríguez, I.G. Fernández, et al., Importance of intense male sexual behavior for inducing the preovulatory LH surge and ovulation in seasonally anovulatory female goats, Theriogenology 82 (2014) 1028–1035 http://dx.doi.org/10.1016/j.theriogenology.2014.07.024.

Sexually active bucks prevent the seasonal decrease of LH in female goats

A.L. Muñoz [a], M. Bedos [b], H. Hernández [a], D. Chesneau [d], G. Duarte [a], J. Vielma [a], L.A. Zarazaga [c], P. Chemineau [d], M. Keller [d], J.A. Delgadillo [a*]

[a]*Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez y Carretera a Santa Fe, C.P. 27054 Torreón, Coahuila, Mexico*

[b]*Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, Mexico*

[c] *Departamento de Ciencias Agroforestales, Universidad de Huelva, Carretera de Palos de la Frontera s/n, 21819, Palos de la Frontera, Huelva, Spain*

[d] *PRC, INRA, CNRS, Université de Tours, IFCE, Agreenium, Nouzilly, France*

* Corresponding author at: Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez y Carretera a Santa Fe, 27054, Torreón, Coahuila, Mexico. Tel: + 52 (871) 729 76 42; fax: + 52 (871) 729 76 42

E-mail address: joaldesa@yahoo.com

Abstract

In goats, the permanent presence of bucks rendered sexually active by photoperiodic treatments, prevents the occurrence of seasonal anovulation, and the introduction of these sexually active bucks, induce ovulations during the seasonal anestrus. We used ovariectomized goats bearing subcutaneous implants filled or not with estradiol to determine 1) whether the permanent presence of sexually active bucks prevents the decrease of LH during the seasonal anestrus, and 2) whether the introduction of photostimulated bucks increases the plasma LH concentrations during the seasonal anestrus. In Experiment 1, we used ovariectomized goats bearing 12-mm subcutaneous implants containing estradiol (OVX+E2). One group of goats (n=13) remained in permanent contact with naturally (November-January) or photostimulated, sexually active bucks (February-May); the other group (n=13) remained in permanent contact with control bucks which displayed intense (November-January) or weak sexual behavior (February-May). Plasma LH concentrations were high and did not differ between groups from November to January ($P>0.05$), when control bucks displayed intense sexual behavior. Afterwards, LH concentrations decreased from March ($P < 0.05$), and remained low until May, when control males displayed weak sexual behavior. On the contrary, LH concentrations did not decrease and remained high from March to May in goats in contact with the photostimulated bucks ($P > 0.05$). In Experiment 2, two groups of females (n=6 each) bearing 12-mm empty subcutaneous implants were exposed, during the seasonal anestrus, to control or photostimulated bucks; two other groups (n=6 each) bearing 12-mm subcutaneous implants filled

with estradiol, were also exposed to control or photostimulated bucks. Plasma LH concentrations did not increase in goats bearing empty implants, when exposed to control (from 2.01 ± 0.26 to 1.98 ± 0.31 ng/mL) or photostimulated bucks (from 2.45 ± 0.29 to 2.42 ± 0.21 ng/mL; $P > 0.05$). In contrast, plasma LH concentrations increased (from 0.97 ± 0.41 to 2.80 ± 0.62 ng/mL) in goats exposed to the photostimulated bucks and bearing estradiol implants ($P < 0.05$). We conclude that the permanent presence of sexually active bucks prevented the decrease of plasma LH concentration in OVX+E2 goats during the seasonal anestrus, and that the introduction of the photostimulated bucks increases the plasma LH concentrations in OVX+E2 goats during the seasonal anestrus.

Keywords: Caprine, Reproductive seasonality, Estradiol feedback, Sexual behavior, Photoperiod.

1. Introduction

Breeds of goats and sheep from subtropical and temperate latitudes display a seasonality of their breeding season [1,2]. In these breeds, the breeding season occurs in autumn and winter, whereas seasonal anovulation occurs in spring and summer [3,4]. This seasonality of reproduction is controlled by photoperiodic variations, which modify the estradiol negative feedback on LH secretion. In fact, the estradiol negative feedback increases during long days, reducing the secretion of LH, inhibiting ovulations to occur, or reducing the plasma LH concentrations in ovariectomized females bearing subcutaneous implants of estradiol-l7 ß (OVX+E2) [3,5,6].

In seasonal breeds of goats and sheep, the introduction of males into a group of seasonally anovulatory females reactivates the secretion of LH, leading to ovulation within the first five days of contact [7–9]. A factor that can modify the response of females exposed to males is the intensity of male sexual behavior. Thus, males submitted to photoperiodic treatments to stimulate their sexual behavior during the non-breeding season, are more efficient to stimulate LH secretion and ovulations, than untreated males displaying low sexual behavior [10,11]. Interestingly, in goats and ewes, the permanent presence of photostimulated males, prevents the seasonal anovulation to occur, and extend their estrus activity during the seasonal anestrus, respectively [12,13]. Considering that the permanent presence of the sexually active males prevents the seasonal anovulation, and that the introduction of the photostimulated bucks stimulates the ovulatory activity in goats during the seasonal anestrus, we

hypothesized that in both cases, the photostimulated bucks are able to reduce or counterbalance the seasonal negative feedback of estradiol on LH secretion. To test this possibility, we did two experiments. In Experiment 1, OVX+E2 remained in permanent presence with control or sexually active bucks from November to May, and we monitored whether the permanent presence of the photostimulated bucks could be able to maintain high LH plasma concentrations despite the inhibitory effect of the increasing days, as it did for maintaining ovulations in entire goats [12]. In Experiment 2, OVX female goats bearing subcutaneous implants with or without estradiol, were suddenly exposed to control or photostimulated bucks during the seasonal anestrus, and we monitored whether the introduction of the photostimulated bucks could be able to increase the plasma LH plasma concentrations despite the inhibitory effect of the increasing days, as it did for inducing ovulations in entire goats [14,15].

2. Materials and methods

2.1 General conditions

The procedures used in these studies were in strict accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals [16]. The two experiments were conducted in the Laguna region (latitude 26° 23´N, longitude 104° 47´ W), State of Coahuila, in northern Mexico. The photoperiod in this region varies from 13 h 41 min of light at the summer solstice, to 10 h 19 min of light at the winter solstice. In both studies, all females and males were fed with 2 kg of alfalfa hay (18% CP) and

200 g of commercial concentrate feed (14% CP; 1.7 Mcal/kg), with water and mineral blocks *ad libitum.* Males and females were kept in shaded open pens. All males used in this study had previous sexual experience. Female goats were three-year old and multiparous. Females were ovariectomized in February to standardize their physiological state according to the method described previously [17,18]. Immediately following ovariectomy, each goat received a silastic subcutaneous implant of 12-mm of long containing crystalline estradiol-17ß (Sigma Chemical Co., Strasbourg; internal diameter 3.35 mm and external diameter 4.65 mm) to avoid an increase of LH secretion due to the absence of endogenous estradiol [3,19].

2.2 Experiment 1

The objective of Experiment 1 was to determine the plasma LH concentrations in OVX+E2 goats that remained in continuous presence of control or sexually active bucks from November to May.

2.2.1 Stimulation of sexual behavior of males by photoperiodic treatments

Control males were subjected to natural photoperiodic conditions (n=8). These males displayed intense sexual behavior from November to January, and low sexual behavior from February to May. Another two groups of males were subjected to natural photoperiodic conditions, and received two subcutaneous ear-implants containing 18 mg of melatonin (MELOVINE® CEVA Santé Animale, France) from July 15th to September 15th (Photostimulated males-1; n=2), or from September 1st to October 31st (Photostimulated males-2; n=2). After

removal of the subcutaneous implants of melatonin, these four bucks were exposed to 2.5 mo of artificial long days as described previously [12]. These photostimulated males displayed an intense sexual behavior 1.5 mo after the end of artificial long days, and during about 2.5 mo [11,12]. Therefore, the photostimulated males-1, were sexually active from mid-January until March, whereas the photostimulated males-2, were sexually active from March until May.

2.2.2 Preparation of females and experimental design

We used 26 OVX+E2 female goats. All these females received a silastic subcutaneous implant of 12-mm containing estradiol. The females were divided into two groups (n=13 each) balanced for body condition score (BCS: 1= very thin, 4= fat; [20]; 3.0 ± 0.2 and 2.9 ± 0.1, respectively; Mean ± SEM). From November 5th to January 31st, both groups of females were in contact with control males, which displayed intense sexual behavior (n=2 each). Then, on February 1st, one group was put in contact with the photostimulated males-1 (n=2), and on March 29th, these males were replaced with the photostimulated males-2, which remained with females until May 21st (n=2). In the other group, control males were replaced by new control ones on the same dates as in the other group, to avoid a possible effect of the introduction of novel males (n=2 each time).

2.2.3 Measurements

In females, plasma LH concentration was determined three times per wk. All blood samples were collected by jugular venipuncture in tubes containing 30 µL of heparin. After collection, the samples were centrifuged immediately at 3500 x g for 30 min, and the plasma samples obtained were stored at -20°C until assayed by RIA, according to Faure et al. [21]. All samples were run in a single assay. Sensitivity was 0.1 ng/mL and the intra-assay CV was 6.2 %.

In bucks, sexual behavior was assessed by direct observation once a month at from 8:00 to 8:15 [22,23]. However, we showed only the results of nudging, because is a reliable variable of the sexual activity of bucks [15].

2.2.4 Statistical Analyses

LH concentrations were analyzed with an ANOVA for repeated measures with two factors (sexual condition of males and time). Comparisons between each point of sampling in females in contact with photostimulated or control bucks in breeding season or seasonal anestrus were analyzed with a two-sample *t*-test. Comparisons of mean LH plasma concentrations between breeding season and seasonal anestrus periods in each group were made through paired *t*-test. The analyses were computed using the statistical package SYSTAT 13 [24]. Differences were considered significant at the level of $P \leq 0.05$.

2.3 Experiment 2

The objective of Experiment 2 was to determine the LH plasma concentration in OVX female goats bearing 12-mm subcutaneous implants containing or not estradiol-l7ß and exposed to control or photostimulated bucks during the seasonal anestrus.

2.3.1 Stimulation of sexual behavior of males by a photoperiodic treatment

The control bucks were subjected to natural photoperiodic conditions, and displayed low sexual behavior during the study (n=2). The bucks were rendered sexually active by exposure to long days as described previously (n=2) [11,25]. Briefly, these bucks were exposed to 2.5 mo of artificial long days (16 h of light/8 h of darkness) from November 1st to January 16th; then, they were exposed to natural variations of day-length until the end of the study. This photoperiodic treatment stimulates testosterone secretion during March and April and, therefore, improves significantly the intensity of their sexual behavior during the non-breeding season [11,25].

2.3.2 Preparation of females

We used OVX female goats (n=24). On April 1st, females were divided into two groups (n=12 each) balanced for body condition score: i) females that received an empty silastic subcutaneous implant of 12-mm (BCS: 2.8 ± 0.1), and ii) females that received silastic subcutaneous implants of 12-mm filled with crystallized estradiol-17ß (BCS: 2.9 ± 0.2; n=12).

2.3.3 Male effect

On April 11 at 13:00, females were exposed to males. Half of each female group (empty or E2 implants) was exposed to control bucks (n=2) or to photostimulated bucks (n=2), resulting on four subgroups: i) OVX goats + control bucks; ii) OVX goats + photostimulated bucks; iii) OVX+E2 goats + control bucks; iv) OVX+E2 goats + photostimulated bucks. Each subgroup was isolated from the others (> 200 m). The bucks remained in contact with does until next day at 19:00.

2.3.4 Measurements

In females, plasma LH concentrations were determined every 15 min from 6 h before (7:00-13:00) to 6 h after (13:15-19:00) the introduction of bucks. Plasma LH concentration was also measured the next day during 6 h every 15 min, from 13:00 to 19:00 to evaluate whether any effect occurring just after the introduction of bucks would remain for a longer duration. All blood samples were collected, and LH was determined as described in Experiment 1. The sensitivity of the LH assay was 0.1 ng/mL and the intra-assay CV was 5.5 %. All samples were run in a single assay.

In bucks, sexual behavior was individually recorded during 15 min (from 13:00 to 13:15) after their introduction into the groups of females, and the next day at the same hour and for the same duration.

2.3.5 Statistical Analyses

With the aim to approximate data to a normal distribution, the statistical analyses were made with logarithm values and presented in real values. Data of plasma LH concentrations were analyzed by an ANOVA for repeated measures with two factors. In addition, we calculated the mean concentration by time windows of 6 h (6 h before introduction of males, 6 h after introduction of males, and 6 h the next day after introduction of males) for each type of implant and comparisons between types of males (control and photostimulated) within each group were made by using an independent *t*-test. The evolution of mean plasma LH concentrations within each group by type of male was analyzed by a paired t-test. Mean LH concentrations before introduction of bucks were compared between different types of implants and types of males using an ANOVA. When significant differences were determined, comparisons between subgroups were carried out using the Fisher LSD post-hoc test. The comparisons of the mean LH concentration from the three time windows into each type of implant were made through paired *t*-test. The analyses were computed using the statistical package SYSTAT 13 [24]. Differences were considered significant at the level of $P \leq 0.05$.

3. Results

3.1 Experiment 1

3.1.1 Plasma concentrations of LH in goats permanently exposed to sexually active vs. sexually inactive bucks

The plasma LH concentrations varied according to time (time effect: $F_{80,1920}$ = 12.095, P = 0.0001). In addition, there was an interaction between time and groups of goats exposed to control or photostimulated males ($F_{80,1920}$ = 7.822, P = 0.0001). The plasma LH concentrations of goats were high and did not differ from November to February, independently if they were exposed to control or photostimulated bucks ($F_{1,24}$ = 0.652, P = 0.428). On the contrary, the plasma LH concentrations differed between groups from March 14th until the end of study ($F_{1,24}$ = 19.472, P = 0.0001). In fact, in goats in contact with control males, plasma LH concentrations decreased from March to May (P = 0.001), whereas in goats in contact with photostimulated males, plasma LH concentrations remained high during the same period (P = 0.101; Figure 1).

3.1.2 Sexual behavior of males

Control males displayed high frequencies of nudging from November to January. Thereafter, the number of nudging decreased in February and March, and none nudging behavior was registered in April and May. On the contrary,

photostimulated males displayed high frequencies of nudging from February to May (Figure 2).

3.2 Experiment 2

3.2.1 Plasma concentrations of LH in goats exposed to sexually active versus inactive bucks

Prior to the introduction of bucks, mean plasma LH concentrations were lower in goats bearing the implants with estradiol (around 2-2.5 ng/mL), than in those bearing the empty implants (around 1 ng/mL, $F_{3,20}$ = 4.370, P = 0.016; Figure 3; Table 1).

During the first 6 h after male introduction, a strong effect of the type of males on LH plasma concentration was observed (control vs photostimulated, $F_{3,15}$ = 6.442, P = 0.020), but not with type of estradiol implants ($F_{3,15}$ = 2.671, P = 0.118). In fact, in goats bearing the empty implant, mean plasma LH concentrations did not differ among those exposed to control or photostimulated males (P = 0.187; Table 1). In contrast, in goats bearing estradiol implants, plasma LH concentrations were higher in females exposed to photostimulated males than in those exposed to control ones (P = 0.047; Table 1).

On the following day after male introduction, plasma LH concentrations in goats bearing empty implant did not differ between females exposed to photostimulated or control males (P = 0.072). In contrast, in goats bearing

estradiol implants, LH concentrations were higher in females exposed to photostimulated males than in those exposed to control males (P = 0.039; Table 1).

In goats bearing the empty implants, the mean LH plasma concentrations did not increase after the introduction of the control or photostimulated males compared with levels registered prior the introduction of males (P = 0.652 and P = 0.746 respectively). However, in these females, the plasma LH concentrations decreased one day after the introduction of the control bucks (P = 0.01; Table 1). Finally, in goats bearing the estradiol implants, the mean LH plasma concentrations were only increased after the introduction of photostimulated but not after control ones (P = 0.047 and P = 0.062, respectively). Those concentrations remained elevated 24 h later in goats exposed to photostimulated males (P = 0.831) and unchanged in females exposed to control males (P = 0.518).

3.2.2 Sexual behavior of males

Sexual behavior of males, appreciated by the mean number of nudgings, when introduced in the groups of females and the following day to introduction, was much higher in photostimulated bucks than in control ones (38 ± 5 vs. 0 in both days).

4. Discussion

In the present studies, we hypothesized that the permanent presence of the photostimulated buck or the sudden introduction of these bucks would counterbalance the negative feedback of estradiol on LH secretion in seasonally anestrus goats, resulting in the maintenance of high plasma LH concentrations, or increasing plasma LH concentrations during the seasonal anestrus. Therefore, in Experiment 1, the continuous presence of -naturally and photostimulated- sexually active males enabled the females to maintain high plasma LH concentrations from March to May, months corresponding to the seasonal anestrus. In contrast, the LH concentrations decreased from March, and remained low until May in females in contact with the control, and therefore, sexually inactive males. In Experiment 2, the introduction of the photostimulated bucks stimulated the LH secretion in OVX+E goats bearing a subcutaneous estradiol implant, but not in those exposed to the control males. Taken together, the present results confirm our initial hypothesis and strongly suggest that the intensity of the sexual behavior of males is an important element to control the seasonal variations of LH secretion in goats, probably by counterbalancing the negative feedback exerted by estradiol on LH secretion.

In Experiment 1, the plasma LH concentrations decreased during the seasonal anestrus in goats in permanent presence of the control bucks. This decrease of LH concentrations occurred when the sexual behavior of control males also decreased. Our findings coincide with previous ones described in OVX+E goats and ewes in contact with males or isolated from them [3,19,26,27]. In our study, the decrease of LH concentrations during the

seasonal anestrus, was probably induced by the installation of the estradiol negative feedback on LH secretion, as reported previously in goats and ewes [28–30]. Contrary to what was observed in females in contact with control males, the seasonal decrease of LH concentrations was prevented by the presence of the photostimulated bucks, which displayed high sexual behavior from February to May. Our findings strongly suggest that the permanent presence of males displaying intense sexual behavior is an important key to prevent the installation of the negative feedback of estradiol on LH secretion, allowing LH concentrations to remain high during the anestrus season. This hypothesis is supported by the fact that the permanent presence of the sexually active males prevents the display of seasonal anovulation in intact goats, whereas the presence of the sexually inactive males did not do so [12]. The effect of permanent presence of the sexually active males is probably exerted at the central level in the nervous system, downstream to the inhibitory effect of photoperiod.

In Experiment 2, the patterns of plasma LH concentrations differed between goats bearing estradiol o empty implants, and exposed to control or photostimulated males. Prior the introduction of control or photostimulated males, LH concentrations was lower in goats bearing the estradiol implants than in those bearing empty implants. The difference between groups on LH concentrations could be directly related to the presence of estradiol. Indeed, in ovariectomized goats or ewes that did not receive any estradiol treatment, the plasma concentrations of LH during the seasonal anestrus are higher than in those bearing estradiol implants [3,30]. Therefore, our findings clearly indicate

that the estradiol implants of 12-mm released enough estradiol to reduce the LH secretion during the seasonal anestrus.

In our study, plasma LH concentration increased significantly in goats bearing the estradiol implants and exposed to the sexually active bucks, but not to control males. The stimulation of LH secretion in goats exposed suddenly to photostimulated bucks occurred probably because these males were able to counterbalance the negative feedback of estradiol on LH secretion, whereas the control males did not do so. Is important to notice that contrary to what was observed in goats bearing the estradiol implants, in goats bearing the empty implant, the LH concentration did not increase after introduction of control or photostimulated bucks. Our results concerning goats bearing the empty implant agree with those reported in ovariectomized ewes exposed to rams [31]. Therefore, it appears that in OVX goats or ewes bearing empty implant, the introduction of males did not stimulate LH secretion because in these females the LH concentrations are already high, due to the absence of a negative feedback of estradiol on LH secretion.

Globally, our findings strongly suggest that only sexually active males can inhibit or dramatically reduce the negative feedback of estradiol on LH secretion during seasonal anestrus. Our results may also suggest that the sexually active males reactivate the gonadotropic axis along the upstream steps of the GnRH neurons, maybe via kisspeptin signaling which directly controls GnRH release in various mammals [32]. Indeed, in goats, a pool of kisspeptinergic neurons located in the arcuate nucleus acts like a pulse generator that controls the release of GnRH at the level of the median eminence [33]. In ewes,

kisspeptinergic neurons of the preoptic area and arcuate nucleus express estrogen receptor alpha [34]. Interestingly, it appears that the kisspeptinergic neurons are implicated in the negative feedback of estrogens in seasonal ewes [35]. Firstly, they observed that the expression of kisspeptin in the arcuate nucleus is decreased when estrogens are administrated, suggesting that the kisspeptinergic neurons mediate the negative feedback of estrogens on GnRH secretion [35]. Secondly, they demonstrated that the expression of kisspeptin in the arcuate nucleus of ewes is increased during the breeding season in comparison with the anestrus season [35,36]. Finally, De Bond et al. [37] demonstrated that the introduction of a ram among a group of anovulatory females induces the activation of GnRH cells in the medial preoptic area and kisspeptin cells in the arcuate nucleus as evidenced by the higher number of cells that expressed the marker of cellular activation c-Fos in GnRH and kisspeptin cells, respectively. A recent study in goats also observed that neurons of the arcuate nucleus are activated by the introduction of a sexually active buck [38]. As a whole, it is likely that the estrogen feedback takes place at the level of kisspeptinergic neurons, and that sexually active bucks are able to activate this pool of neurons to counterbalance the enhanced negative feedback of estrogens during the seasonal anestrus in goats.

We conclude that in OVX+E2 goats, the permanent presence of the sexually active males maintain high the plasma LH concentrations during the seasonal anestrus, and that the sudden introduction of these sexually active males increases the plasma LH concentrations during the seasonal anestrus. These findings strongly suggest that the sexually active males can significantly

counterbalance the negative feedback of estradiol on LH secretion. The current results and those of Delgadillo et al. [12] increase our knowledge concerning the mechanisms involved in the control of the annual rhythm of reproduction of goats, mainly, to the importance of the intensity of the sexual behavior of males.

Acknowledgments

We thank to José Alfredo Flores, Gonzalo Fitz-Rodríguez, and Ilda Fernández for their technical support during the study. We also thank to Benoît Malpaux and Alain Caraty for their contribution in the preparation of the experimental design of our studies. We are grateful to Dolores López and Esther Peña for their kind secretarial and administrative support, and to Jesús García and Sergio Daniel Delgadillo for taking care of experimental animals. The authors are also grateful to Anne-Lyse Lainé and the whole hormonal assay platform in Nouzilly, France for hormonal determinations. These studies were funded by grants from the CONACYT-Ciencia Básica (254176); CONACYT (Mexico)-ANR (France; 159884), and SEP-CONACYT-ANUIES-ECOS (M13A01) programs. A.L. Muñoz was supported by a scholarship of CONACYT during his doctorate studies. This research was conducted as part of the CABRAA International Associated Laboratory between Mexico (UAAAN-CIRCA) and France (INRA-PRC).

References

[1] Ortavant R, Pelletier J, Ravault JP, Thimonier J, Volland P. Photoperiod: main proximal and distal factor of the circannual cycle of reproduction in farm mammals. Oxf Rev Reprod Biol 1985;7:305–45.

[2] Delgadillo JA. Environmental and social cues can be used in combination to develop sustainable breeding techniques for goat reproduction in the subtropics. Animal 2011;5:74–81.

[3] Duarte G, Flores JA, Malpaux B, Delgadillo JA. Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability. Domest Anim Endocrinol 2008;35:362–70.

[4] Chanvallon A, Sagot L, Pottier E, Debus N, François D, Fassier T, Scaramuzzi RJ, Fabre-Nys C. New insights into the influence of breed and time of the year on the response of ewes to the “ram effect.” Animal 2011;5:1594–604.

[5] Karsch FJ, Bittman EL, Foster DL, Goodman RL, Legan SJ, Robinson JE. Neuroendocrine basis of seasonal reproduction. Recent Prog Horm Res 1984;40:185–232.

[6] Barrell GK, Thrun LA, Brown ME, Viguie C, Karsch FJ. Importance of photoperiodic signal quality to entrainment of the circannual reproductive rhythm of the ewe. Biol Reprod 2000;63:769–74.

[7] Martin GB, Oldham CM, Cognié Y, Pearce DT. The physiological responses of anovulatory ewes to the introduction of rams — A review.

Livest Prod Sci 1986;15:219–47.

[8] Chemineau P. Possibilities for using bucks to stimulate ovarian and oestrous cycles in anovulatory goats - a review. Livest Prod Sci 1987;17:135–47.

[9] Delgadillo JA, Gelez H, Ungerfeld R, Hawken P A R, Martin GB. The “male effect” in sheep and goats-Revisiting the dogmas. Behav Brain Res 2009;200:304–14.

[10] Bedos M, Duarte G, Flores JA, Fitz-Rodríguez G, Hernández H, Vielma J, Fernández IG, Chemineau P, Keller M, Delgadillo JA. Two or 24 h of daily contact with sexually active males results in different profiles of LH secretion that both lead to ovulation in anestrous goats. Domest Anim Endocrinol 2014;48:93–9.

[11] Ponce JL, Velázquez H, Duarte G, Bedos M, Hernández H, Keller M, Chemineau P, Delgadillo JA. Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of male goats to stimulate ovulatory activity in seasonally anovulatory females. Domest Anim Endocrinol 2014;48:119–25.

[12] Delgadillo JA, Flores JA, Hernández H, Poindron P, Keller M, Fitz-Rodríguez G, Duarte G, Vielma J, Fernández IG, Chemineau P. Sexually active males prevent the display of seasonal anestrus in female goats. Horm Behav 2015;69:8–15.

[13] Abecia JA, Chemineau P, Flores JA, Keller M, Duarte G, Forcada F, Delgadillo JA. Continuous exposure to sexually active rams extends estrous activity in ewes in spring. Theriogenology 2015;84:1549–55.

[14] Loya-Carrera J, Bedos M, Ponce-Covarrubias JL, Hernández H, Chemineau P, Keller M, Delgadillo JA. Switching photo-stimulated males between groups of goats does not improve the reproductive response during the male effect. Anim Reprod Sci 2014;146:21–6.

[15] Muñoz AL, Bedos M, Aroña RM, Flores JA, Hernández H, Moussu C, Briefer EF, Chemineau P, Keller M, Delgadillo JA. Efficiency of the male effect with photostimulated bucks does not depend on their familiarity with goats. Physiol Behav 2016;158:137–42.

[16] Secretaría de agricultura, ganadería, desarrollo rural pesca y, alimentación. Norma Oficial Mexicana NOM-062-ZOO-1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Diario oficial de la federación, Ciudad de México, D.F., 22 de agosto de 2001.

[17] Goodman RL, Bittman EL, Foster DL, Karsch FJ. Alterations in the control of luteinizing hormone pulse frequency underlie the seasonal variation in estradiol negative feedback in the ewe. Biol Reprod 1982;27:580–9.

[18] Montgomery GW, Martin GB, Pelletier J. Changes in LH secretion after ovariectomy in Ile-de-France ewes in two seasons. J Reprod Fertil 1985;73:173–83.

[19] Malpaux B, Robinson JE, Brown MB, Karsch FJ. Importance of changing photoperiod and melatonin secretory pattern in determining the length of the breeding. J Reprod Fertil 1988;83:461–70.

[20] Walkden-Brown SW, Restall BJ, Scaramuzzi RJ, Martin GB, Blackberry MA. Seasonality in male Australian cashmere goats: Long term effects of

castration and testosterone or oestradiol treatment on changes in LH, FSH and prolactin concentrations, and body growth. Small Rumin Res 1997;26:239–52.

[21] Faure MO, Nicol L, Fabre S, Fontaine J, Mohoric N, McNeilly A, Taragnat C. BMP-4 inhibits follicle-stimulating hormone secretion in ewe pituitary. J Endocrinol 2005;186:109–21.

[22] Gonzalez R, Poindron P, Signoret JP. Temporal variation in LH and testosterone responses of rams after the introduction of oestrous females during the breeding season. J Reprod Fertil 1988;83:201–8.

[23] Flores JA, Véliz FG, Pérez-Villanueva JA, Martínez de la Escalera G, Chemineau P, Poindron P, Malpaux B, Delgadillo JA. Male reproductive condition is the limiting factor of efficiency in the male effect during seasonal anestrus in female goats. Biol Reprod 2000;62:1409–14.

[24] Product of Cranes Software International Ltd. SYSTAT 13 2009.

[25] Bedos M, Flores JA, Fitz-Rodríguez G, Keller M, Malpaux B, Poindron P, Delgadillo JA. Four hours of daily contact with sexually active males is sufficient to induce fertile ovulation in anestrous goats. Horm Behav 2010;58:473–7.

[26] Legan SJ, Karsch FJ. Photoperiodic control of seasonal breeding in the ewes: modulation of the negative feedback action of estradiol. Biol Reprod 1980;23:1061–8.

[27] Legan SJ, Karsch FJ. Importance of retinal photoreceptors to the photoperiodic control of seasonal in the ewe. Biol Reprod 1983;29:316–25.

[28] Dardente H. Melatonin-dependent timing of seasonal reproduction by the Pars Tuberalis: pivotal roles for long daylengths and thyroid hormones. J Neuroendocrinol 2012;24:249–66.

[29] Mori Y, Tanaka M, Maeda K, Hoshino K, Kano Y. Photoperiodic modification of negative and positive feedback effects of oestradiol on LH secretion in ovariectomized goats. J Reprod Fertil 1987;80:523–9.

[30] Legan SJ, Karsch FJ, Foster DL. The endocrine control of seasonal reproductive function in the ewe: A marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion. Endocrinology 1977;101:818–24.

[31] Martin GB, Scaramuzzi RJ, Lindsay DR. Effect of the introduction of rams during the anoestrous season on the pulsatile secretion of LH in ovariectomized ewes. J Reprod Fertil 1983;67:47–55.

[32] Caraty A, Lomet D, Sébert ME, Guillaume D, Beltramo M, Evans NP. Gonadotrophin-Releasing Hormone release into the hypophyseal portal blood of the ewe mirrors both pulsatile and continuous intravenous infusion of Kisspeptin: an insight into Kisspeptin's mechanism of action. J Neuroendocrinol 2013;25:537–46.

[33] Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H. Neurokinin B and Dynorphin A in Kisspeptin neurons of the Arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile Gonadotropin-Releasing Hormone secretion in the goat. J Neurosci 2010;30:3124–32.

[34] Franceschini I, Lomet D, Cateau M, Delsol G, Tillet Y, Caraty A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. Neurosci Lett 2006;401:225–30.

[35] Smith JT, Clay CM, Caraty A, Clarke IJ. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology 2007;148:1150–7.

[36] Clarke IJ, Smith JT, Caraty A, Goodman RL, Lehman MN. Kisspeptin and seasonality in sheep. Peptides 2009;30:154–63.

[37] De Bond JP, Li Q, Millar RP, Clarke IJ, Smith JT. Kisspeptin signaling is required for the luteinizing hormone response in anestrous ewes following the Introduction of males. PLoS One 2013;8:1–11.

[38] Bedos M, Portillo W, Dubois JP, Duarte G, Flores JA, Chemineau P, Keller M, Paredes RG, Delgadillo JA. A high level of male sexual activity is necessary for the activation of the medial preoptic area and the arcuate nucleus during the “male effect” in anestrous goats. Physiol Behav 2016;165:173–8.

Table 1. Plasma LH concentrations (ng/mL; Mean ± SEM) of OVX female goats bearing subcutaneous implants containing or not estradiol 17-β estradiol, and exposed to control or photostimulated bucks. Control bucks remained under natural photoperiodic conditions and displayed low sexual behavior during the study. Photostimulated bucks were rendered sexually active by exposure to 2.5 mo of long days from November 1st (16 h of light per day), followed by natural photoperiodic conditions, and displayed high sexual behavior during the study.

Group of females	Group of males	LH before introduction of males (hour -6 to 0)	LH after introduction of males (hour 0 to 6)	LH next day after introduction of males (hour 24 to 30)
Empty implants	Control males	[a] 2.01 ± 0.26	[c] 1.98 ± 0.31	[c] 1.46 ± 0.21 *
	Photostimulated males	[a] 2.45 ± 0.29	[c] 2.42 ± 0.21	[c] 1.90 ± 0.12
Estradiol implants	Control males	[b] 1.04 ± 0.44	[d] 1.29 ± 0.46	[d] 1.29 ± 0.45
	Photostimulated males	[b] 0.97 ± 0.41 *	[e] 2.80 ± 0.62	[e] 2.58 ± 0.60

[a,b]Different letters indicate significant difference between groups of femeales before the introduction of bucks (P<0.05; Fisher LSD test).

[c,d,e]Different letters indicate significant difference between groups of females bearing different type of implants, and exposed to control or photostimulated bucks (P<0.05; Two simple t-test)

*Indicate significant difference within the same rows (P<0.05; Paired t-test)

Figure legends

Figure 1. Plasma LH concentrations (ng/mL; Mean ± SEM) of OVX goats bearing 12-mm subcutaneous implants containing estradiol-17 β. Both groups of goats remained in permanent contact with control bucks from November to January, when displayed high sexual behavior. Afterwards, from February to May, one group of goats continued in contact with control bucks, which displayed low sexual behavior (○), whereas the other group was put in permanent contact with the photostimulated bucks, which displayed high sexual behavior from February to May (●). Photostimulated received subcutaneous melatonin ear-implants during 2 months, followed by exposure to 2.5 mo of long days from November 1st (16 h of light per day), and natural photoperiod conditions.

*Indicate significant difference between groups in each sampling point.

Figure 2. Mean (± SEM) monthly number of nudging per test of 15 min of control and photostimulated bucks (n= 2 each month). Control males displayed high sexual behavior from November to January, which decreased from February to May (□). Photostimulated bucks displayed high sexual behavior from February to May (■). Photostimulated bucks were rendered sexually active by exposure to 2.5 mo of long days from November 1st (16 h of light per day), followed by natural photoperiod conditions.

Figure 3. Plasma LH concentrations (ng/mL; Mean ± SEM) of OVX female goats bearing 12-mm subcutaneous implants containing or not estradiol-17 β, and exposed to control (○) or photostimulated bucks (●). Control bucks remained under natural photoperiodic conditions and displayed low sexual behavior during the study. Photostimulated bucks were rendered sexually active by exposure to 2.5 mo of long days from November 1st (16 hours of light per day), followed by natural photoperiod conditions, and displayed high sexual behavior during the study.

↓ Indicate the moment of introduction of males in each group.

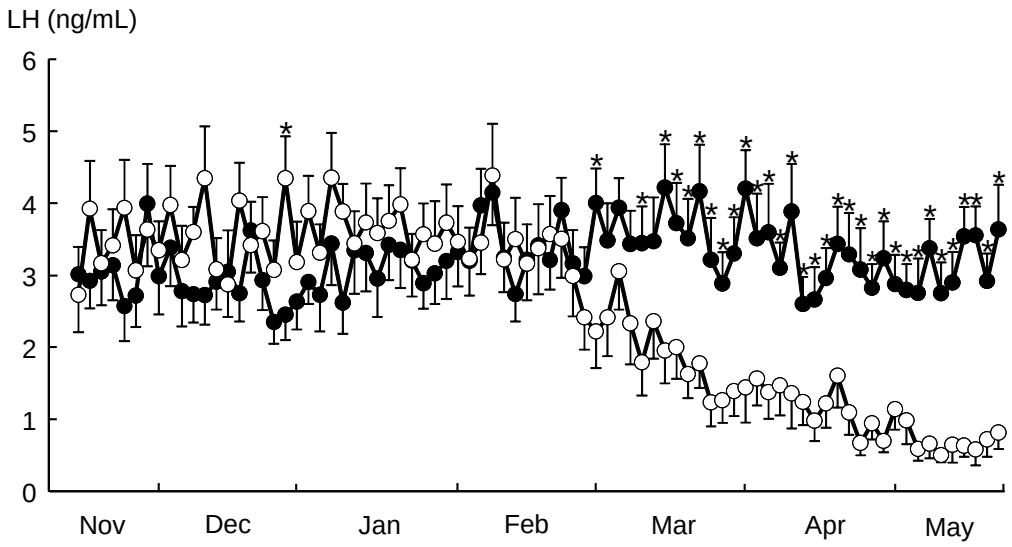

Figure 1

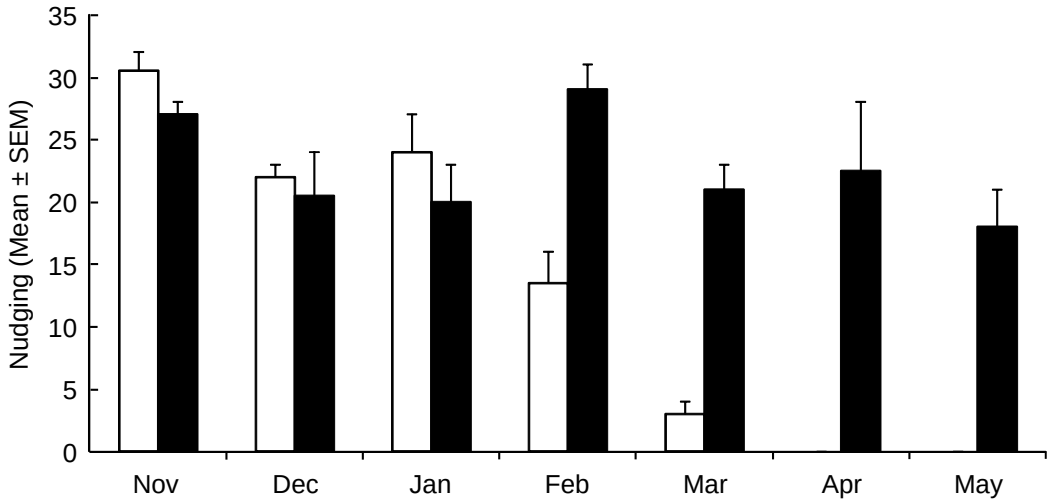

Figure 2

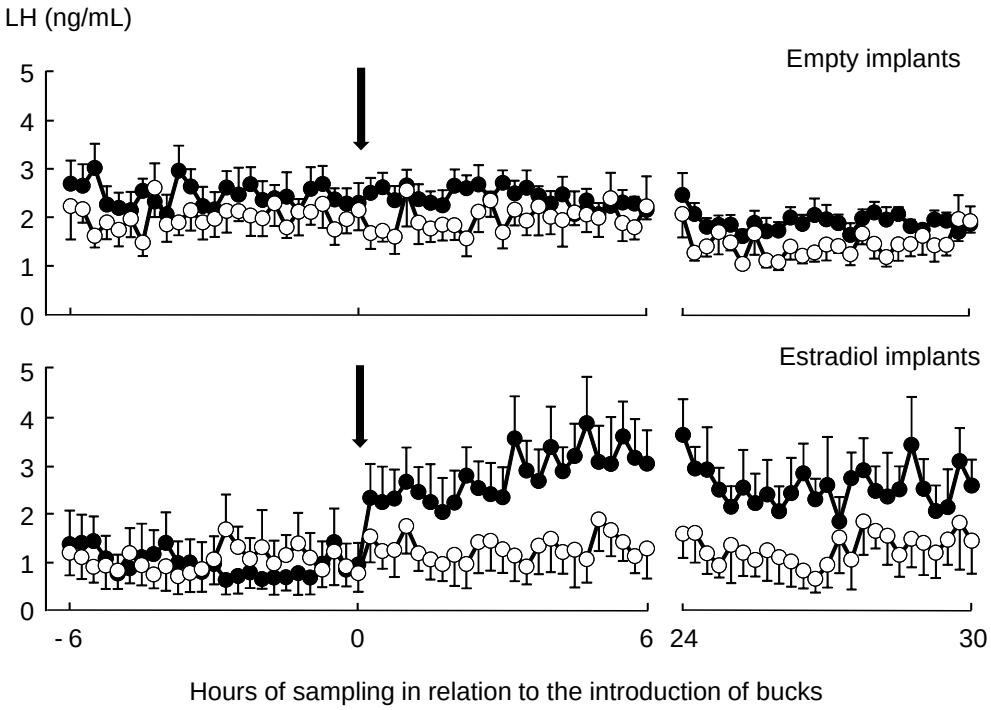
LH (ng/mL)
Empty implants
Estradiol implants
5
4
3
2
1
0
- 6
0
6
24
30
Hours of sampling in relation to the introduction of bucks

Figure 3