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LA PROGESTERONA REDUCE LA PROPORCIÓN DE CICLOS OVULATORIOS CORTOS EN CABRAS ANÉSTRICAS SOMETIDAS AL EFECTO MACHO EN UN SISTEMA DE PRODUCCIÓN INTENSIVO O EXTENSIVO

Tesis

Que presenta JENIFER DENISSE ANDRADE ESPARZA
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LA PROGESTERONA REDUCE LA PROPORCIÓN DE CICLOS OVULATORIOS CORTOS EN CABRAS ANÉSTRICAS SOMETIDAS AL EFECTO MACHO EN UN SISTEMA DE PRODUCCIÓN INTENSIVO O EXTENSIVO

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Se realizaron dos experimentos con el fin de determinar i) si 25 mg de progesterona reducen la proporción de ciclos ovulatorios cortos en cabras expuestas al efecto macho en un sistema de producción extensivo, ii) si dosis menores a 5 mg de progesterona reducen la proporción de ciclos ovulatorios cortos en cabras expuestas al efecto macho. Experimento 1: Las hembras del grupo extensivo (n=45, grupo extensivo+P4), y las de un grupo intensivo (n=45, grupo intensivo+P4) se trataron con 25 mg de progesterona. Otro grupo en intensivo no recibió tratamiento (n=25, grupo intensivo control). La proporción de hembras que presentó ciclo ovulatorio corto fue mayor en el grupo intensivo control (76%) que en el intensivo+P4 (27%) y extensivo+P4 (25%; $P<0.001$). Experimento 2: las hembras de los grupos experimentales recibieron 1 mg (n=15), 3 mg (n=16), 5 mg (n=15) o 25 mg (n=16) de progesterona. Las cabras control no se trataron (n=9). La proporción de cabras que presentaron ciclos ovulatorios cortos fue mayor en las cabras control (78%), aquellas tratadas con 1 mg (85%), 3 mg (50%) y 5 mg (71%), que en las tratadas con 25 mg de progesterona (12%; $P<0.05$). Se concluye que i) el sistema de producción extensivo no modifica la proporción de cabras que presentan ciclos ovulatorios cortos en hembras tratadas con progesterona y expuestas al efecto macho; y ii) que la aplicación de 1, 3 o 5 mg de progesterona no reducen la proporción de cabras que presentan ciclos ovulatorios cortos al exponerlas al efecto macho.

Palabras clave. *caprinos, estacionalidad sexual, ovulación, interacciones socio-sexuales*

ABSTRACT

PROGESTERONE REDUCES THE PROPORTION OF SHORT OVULATORY CYCLES IN ANESTRIC GOATS SUBJECTED TO THE MALE EFFECT IN AN INTENSIVE OR EXTENSIVE PRODUCTION SYSTEM

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Two experiments were conducted to determine i) whether 25 mg of progesterone reduces the proportion of short ovulatory cycles in goats exposed to the male effect in an extensive production system, ii) if doses less than 5 mg of progesterone reduce the proportion of short ovulatory cycles in goats exposed to the male effect. Experiment 1: the females of the extensive group (n=45, extensive+P4 group), and those of an intensive group (n=45, intensive+P4 group) were treated with 25 mg of progesterone. Another intensive group did not receive treatment (n=25, intensive control group). The proportion of females that presented a short ovulatory cycle was greater in the intensive control group (76%) than in the intensive+P4 (27%) and extensive+P4 groups (25%, P<0.001). Experiment 2: females of the experimental groups received 1 mg (n=15), 3 mg (n=16), 5 mg (n=15) or 25 mg (n=16) of progesterone. Control goats were not treated (n=9). The proportion of goats that had short ovulatory cycles was higher in control goats (78%), those treated with 1 mg (85%), 3 mg (50%) and 5 mg (71%), than in those treated with 25 mg of progesterone (12%, P<0.05). It is concluded that i) the extensive production system does not modify the proportion of goats that present short ovulatory cycles in females treated with progesterone and exposed to the male effect; and ii) the application of 1, 3 or 5 mg of progesterone does not reduce the proportion of goats that have short ovulatory cycles when exposed to the male effect.

Keywords. *goats, sexual seasonality, ovulation, socio-sexual interactions*

1. INTRODUCCIÓN

En las razas de cabras que presentan estacionalidad reproductiva, la introducción de un macho cabrío en un grupo de hembras anovulatorias durante el anestro estacional, estimula el comportamiento estral y la ovulación en los primeros 5 días de contacto con los machos. Esta técnica de bioestimulación sexual se le conoce como “efecto macho” (Shelton, 1960). La respuesta sexual de las cabras expuestas al efecto macho depende de la intensidad del comportamiento desplegado por los machos. En efecto, los machos cabríos inducidos a un intenso comportamiento sexual durante el periodo de reposo sexual, al someterlos a días largos artificiales, son más eficaces que los machos no tratados que despliegan un débil comportamiento sexual para estimular la actividad sexual de las cabras durante el anestro estacional (Delgadillo *et al.,* 2002). Después de la primera ovulación en las cabras expuestas al efecto macho, el cuerpo lúteo es de corta duración, por lo que la mayoría de las cabras desarrollan un ciclo ovulatorio corto que dura entre 5 y 10 días. Esta primera ovulación no se asocia en todas las hembras con un comportamiento estral, por lo que el porcentaje de hembras gestantes es bajo (Chemineau *et al.,* 2006). Después de la luteólisis, las cabras ovulan nuevamente, y la fase lútea resultante es de duración normal, es decir, de 14 a 16 días. Esta segunda ovulación es acompañada de comportamiento estral, por lo que el porcentaje de hembras gestantes es elevado (Chemineau *et al.,* 2006). Por lo tanto, el periodo de cubriciones en las cabras expuestas al efecto macho debe extenderse por lo menos durante 15 días para obtener tasas de gestaciones de alrededor del 70% (Araya *et al.,* 2016). Además, si se desea inseminar a las cabras expuestas a los machos, es necesario detectar el estro e inseminar en el segundo estro que manifieste la hembra (Restall, 1988; Baril *et al.,* 1993a). Todo esto dificulta el manejo reproductivo del hato. En cabras, la aplicación de una dosis de 5 o 25 mg de progesterona por vía intramuscular 2 días antes o al momento de la introducción de los machos en el grupo de hembras anéstricas, reduce de manera significativa el porcentaje de ciclos ovulatorios de corta duración, y permite que la primera ovulación se acompañe de un comportamiento estral (Lassoued *et al.,* 1995; Gonzalez-Bulnes *et al.,* 2006; Véliz *et al.,* 2009).

1.1. Justificación

Considerando que, en cabras, la progesterona se ha usado principalmente en hembras estabuladas y bien alimentadas, y que una dosis de 5 mg de progesterona reduce la proporción de hembras que presentan ciclos ovulatorios cortos, el primer objetivo de esta tesis fue determinar si en cabras mantenidas en un sistema de producción extensivo, 25 mg de progesterona reducen la proporción de hembras que presentan ciclos ovulatorios cortos al ser expuestas al efecto macho. El segundo objetivo fue determinar si dosis menores a 5 mg de progesterona reducen la proporción de cabras que presentan ciclos ovulatorios cortos al ser expuestas al efecto macho en condiciones intensivas.

2. REVISIÓN DE LITERATURA

2.1. Estacionalidad reproductiva

La estacionalidad reproductiva resulta de una estrategia evolutiva desarrollada por varias especies de animales silvestres, la cual asegura la supervivencia de la descendencia. Dicha estacionalidad es un mecanismo de adaptación al medio ambiente en las latitudes templadas, el cual garantiza que tanto el nacimiento de las crías como la lactación, se produzcan en primavera-verano, época del año cuando existe mayor disponibilidad de alimento, así como temperaturas ambientales más cálidas (Marshall, 1936; Karsch *et al.,* 1984; Martin *et al.,* 1994; Thiéry *et al.,* 2002; Chemineau *et al.,* 2008).

A pesar de la selección artificial ejercida por el hombre, algunas razas de especies domésticas, como los caprinos (*Capra hircus*) y ovinos (*Ovis aries*) originarias de latitudes templadas (>40°) y subtropicales (23-40º), siguen presentando estacionalidad en su actividad sexual y reproductiva (Thiéry *et al.,* 2002; Chemineau *et al.,* 2007; Delgadillo y Martin, 2015).

2.1.1. Estacionalidad reproductiva en cabras y ovejas

Las cabras y ovejas de algunas razas originarias o adaptadas a latitudes subtropicales se clasifican como poliéstricas estacionales. En las cabras y ovejas, el período de actividad sexual, determinado por la manifestación de comportamiento estral y actividad ovulatoria, ocurre cuando la cantidad de horas luz del día se reduce, es decir, en otoño e invierno, independientemente del hemisferio donde se encuentren los animales (Hafez, 1952; Duarte *et al.*, 2008). Durante el periodo de actividad sexual, las cabras no gestantes manifiestan ciclos estrales y ováricos en promedio cada 21 días (rango: 17 a 26 días), clasificados como ciclos de duración normal (Chemineau *et al.,* 1992). Sin embargo, existen también ciclos cortos (<17 días; promedio 8 días) y largos (>26 días; promedio 39 días; Chemineau *et al.,* 1992; Baril *et al.,* 1993a).

Además, en las cabras es común que se presentan estros sin ovulación al inicio de la estación sexual, y ovulaciones sin estro al final de la misma. Finalmente, el anestro estacional se caracteriza por la ausencia de estros y ovulaciones, y ocurre cuando la cantidad de horas luz del día se incrementa, es decir, en primavera y verano (Duarte *et al.,* 2008; Delgadillo *et al.,* 2015).

2.1.2. Estacionalidad reproductiva en machos cabríos y ovinos

Los machos cabríos y ovinos de razas originarias o adaptadas a latitudes subtropicales presentan también estacionalidad en su actividad sexual. En estos machos, la estación sexual, que se caracteriza por un incremento en las concentraciones plasmáticas de gonadotropinas (LH y FSH) y testosterona, mayor volumen testicular, intenso comportamiento sexual y elevada producción espermática cuantitativa y cualitativa, se desarrolla en verano y otoño (Walkden-Brown *et al.,* 1994; Pérez-Clariget *et al.,* 1998; Delgadillo *et al.,* 1999). En cambio, los valores de las variables antes mencionadas disminuyen durante el periodo de reposo sexual, el cual se desarrolla en invierno y primavera (Walkden-Brown *et al.,* 1994; Pérez-Clariget *et al.,* 1998; Delgadillo *et al.,* 1999).

En conjunto, estos datos indican que los machos cabríos y ovinos de latitudes subtropicales presentan variaciones estacionales en su actividad sexual.

2.2. El fotoperiodo sincroniza la estacionalidad sexual de los caprinos de latitudes subtropicales

Los animales utilizan diversas señales externas que les permiten anticipar y/o adaptarse a los cambios medioambientales que ocurren en las diferentes estaciones del año (Ortavant *et al.,* 1985). En caprinos y ovinos, la principal señal medioambiental que controla la estacionalidad reproductiva es el fotoperiodo, es decir, las variaciones diarias en las horas luz que ocurren anualmente, el cual sincroniza el inicio y final de la estación sexual (Yeates *et al.,* 1947; Lincoln y Short, 1980; Karsch *et al.,* 1984; Malpaux *et al.,* 1987;

Delgadillo *et al.,* 2011). En los caprinos y ovinos, el papel del fotoperiodo se demostró al someter a los animales a cambios alternados entre días largos (14 o 16 h luz/día) y cortos (10 u 8 h luz/día) cada dos o tres meses. En estos diseños experimentales, la actividad ovulatoria y el incremento de las concentraciones plasmáticas de testosterona ocurren únicamente durante los días cortos (Lincoln y Short, 1980; Karsch *et al.,* 1984; Delgadillo y Chemineau, 1992; Delgadillo *et al.,* 2004; Duarte *et al.,* 2010). Por lo tanto, estos datos muestran que el fotoperiodo es el principal factor del medio ambiente que sincroniza la actividad sexual anual de los caprinos.

2.3. Técnicas de control reproductivo para modificar la estacionalidad reproductiva de los caprinos

Se han desarrollado diversas técnicas de control reproductivo para posibilitar la inducción del estro y la ovulación durante el anestro estacional de las cabras y ovejas. La modificación de la estacionalidad reproductiva permite que los partos ocurran fuera de la estación normal y, por tanto, producir leche y carne en los meses en que escasean estos productos. Entre las técnicas que se han utilizado para tal fin, podemos mencionar los tratamientos hormonales, la manipulación del fotoperiodo, y/o las relaciones socio-sexuales, particularmente el “efecto macho”.

2.3.1. Tratamientos hormonales

Los tratamientos hormonales para inducir y/o sincronizar la actividad sexual de las cabras durante el anestro estacional se basan principalmente en el uso de esponjas vaginales impregnadas con progesterona o progestágenos, prostaglandinas y la gonadotropina coriónica equina (eCG), que tiene un efecto similar a la LH y FSH. Las esponjas se insertan durante 10 días en la vagina, y 48 horas antes de retirarlas se aplica prostaglandina y eCG. Este tratamiento induce la ovulación entre 24 y 72 horas después de la aplicación de la eCG (Baril *et al.,* 1993b; Simões, 2015).

2.3.2. Tratamientos fotoperiódicos

Como ya se mencionó anteriormente, el fotoperiodo es el factor principal del medio ambiente que sincroniza la estación sexual de los caprinos, por lo que los tratamientos fotoperiódicos pueden utilizarse para modificar esta estacionalidad sexual (Chemineau *et al.,* 1992; Delgadillo *et al.,* 2004; Duarte *et al.,* 2010). En los machos cabríos del norte de México, por ejemplo, 2.5 meses de días largos (16 h luz/día), seguidos del fotoperiodo natural estimula la secreción de testosterona en marzo y abril, durante el reposo sexual. En consecuencia, los machos despliegan un intenso olor y comportamiento sexual en estos meses (Delgadillo *et al.,* 2002; Ponce *et al.,* 2014; Bedos *et al.,* 2016). De la misma manera, en las hembras caprinas, 2 o 3 meses de días largos (16 h de luz/día) seguidos de días cortos (8 h de luz/día) o decrecientes, estimulan los estros y ovulaciones durante el anestro estacional (Chemineau *et al.,* 1992).

2.3.3. Efecto macho

Otra alternativa para modificar la estacionalidad sexual de los caprinos y ovinos es la manipulación de las interacciones socio-sexuales entre machos y hembras, particularmente la técnica de bioestimulación conocida como “efecto macho” (Shelton, 1960; Gonzalez-Bulnes *et al.,* 2006; Hawken y Martin, 2012; Jorre de St Jorre *et al.,* 2014). De esta forma, la introducción de un macho en un grupo de hembras anéstricas, estimula la secreción de las gonadotropinas (LH y FSH), así como las actividades estral y ovulatoria en los primeros 5 días de contacto entre los géneros (Chemineau, 1987; Delgadillo *et al.,* 2004; Hawken y Martin, 2012).

Es importante subrayar que los machos ovinos y caprinos inducidos a una intensa actividad sexual durante el periodo de reposo, al someterlos a un tratamiento de días largos artificiales, son más eficaces que los machos no tratados para estimular la actividad sexual de las cabras anéstricas mantenidas estabuladas o en condiciones de pastoreo extensivo (Rivas-Muñoz *et al.,* 2007; Delgadillo, 2011; Abecia *et al.,* 2017).

2.3.3.1. Respuesta estral y ovulatoria al efecto macho

La introducción del macho en el grupo de hembras aumenta inmediatamente la secreción pulsátil de las gonadotropinas, las cuales estimulan los folículos ováricos para que secreten estradiol 17-β, lo que desencadena el comportamiento de estro y la aparición del pico preovulatorio de LH alrededor de 53 horas después de iniciado el estro (Chemineau, 1985; Martin *et al.,* 1986; Chemineau, 1987). Por tanto, la primera ovulación ocurre alrededor de 67 horas después de iniciado el pico preovulatorio de LH, por lo que la mayoría de las cabras ovulan en los primeros 3-5 días después del primer contacto con el macho (Chemineau, 1985; Martin *et al.,* 1986; Delgadillo *et al.,* 2006).

En cabras, esta primera ovulación no se asocia a un comportamiento de estro en un número variable de hembras (40-60%). Además, el cuerpo lúteo resultante de la primera ovulación es de corta duración, por lo que la mayoría de las cabras (58-77%) desarrolla un ciclo ovulatorio de corta duración (5-10 días), por lo que el porcentaje de hembras gestantes es muy bajo.

Después de la regresión del cuerpo lúteo, las cabras ovulan nuevamente, y la vida media del cuerpo lúteo de esta segunda ovulación es de duración normal, por lo que las cabras desarrollan un ciclo ovulatorio normal, es decir, de 21 días.

Asimismo, esta segunda ovulación es acompañada de comportamiento estral, por lo que el porcentaje de hembras gestantes es elevado (Chemineau *et al.,* 2006).

2.4. Uso de progesterona para disminuir la manifestación de ciclos estrales y ovulatorios de corta duración

En caprinos y ovinos, la progesterona puede reducir o evitar los ciclos estrales y ovulatorios de corta duración. En efecto, la aplicación de una dosis de 20 o 25 mg de progesterona por vía intramuscular dos días antes o al momento de la introducción de los machos en el grupo de hembras anéstricas, reduce de manera significativa el porcentaje de ciclos ovulatorios de corta duración, y permite que la primera ovulación se acompañe de un comportamiento estral (Oldham *et al.,* 1985; Lassoued *et al.,* 1995; Gonzalez-Bulnes *et al.,* 2006). La efectividad de la progesterona para reducir los ciclos cortos parece depender de la dosis aplicada. En ovinos, por ejemplo, el 46% de las hembras no tratadas con progesterona presentan ciclos cortos al ser expuestas a los machos. Sin embargo, la proporción de hembras que presentan ciclos cortos se reduce al 15%, 12% y 0% en hembras tratadas con 5, 10 y 20 mg de progesterona, respectivamente (Oldham *et al.,* 1985). De manera similar, el 80% de las cabras no tratadas con progesterona presentan ciclos cortos al ser expuestas a los machos. Sin embargo, esta frecuencia se reduce al 20 y 0% en las hembras tratadas con 25 o 5 mg de progesterona, respectivamente (Gonzalez-Bulnes *et al.,* 2006; Véliz *et al.,* 2009). En las cabras tratadas con progesterona, los estros y las ovulaciones se presentan durante los primeros 6 días después de la introducción de los machos (Lassoued *et al.,* 1995; Gonzalez-Bulnes *et al.,* 2006; López-Sebastian *et al.,* 2007). Estos datos muestran que la progesterona reduce o evita los ciclos ovulatorios cortos, y permite una mejor sincronización sexual de las cabras expuestas a los machos.

2.5. Sistema de producción extensivo en la Comarca Lagunera de Coahuila

En la Comarca Lagunera, la mayoría (90%) de los caprinos locales se mantienen en un sistema de producción extensivo, en el cual los animales se alimentan sólo de la flora natural de los agostaderos, sin recibir un complemento alimenticio en el corral. La disponibilidad de la vegetación natural que consumen los caprinos disminuye drásticamente de noviembre a marzo (Sáenz-Escárcega *et al.,* 1991).

En el sistema de producción extensivo de la Comarca Lagunera, los animales salen al campo en la mañana y regresan en la tarde. En la noche son alojados en instalaciones abiertas. Generalmente, las hembras permanecen todo el año junto con los machos, y cuando esto sucede, el 80% de los partos ocurren de noviembre a marzo, lo que sugiere que la mayoría de las gestaciones ocurren entre junio y octubre.

Las crías son amamantadas por las madres, destetadas y vendidas entre las 4 y 6 semanas de edad. Sin embargo, la venta de leche es considerada el ingreso más importante que tienen los productores (Sáenz-Escárcega *et al.,* 1991).

3. OBJETIVOS

1. El primer objetivo de esta tesis fue determinar si en cabras en un sistema de producción extensivo y expuestas al efecto machos, 25 mg de progesterona reducen la proporción de hembras que presentan ciclos ovulatorios cortos.

2. El segundo objetivo de esta tesis fue determinar si en cabras expuestas a los machos, 1 o 3 mg de progesterona reducen la proporción de cabras que presentan ciclos ovulatorios cortos.

4. HIPÓTESIS

1. En cabras en un sistema de producción extensivo y expuestas al efecto macho, 25 mg de progesterona reducen la proporción de hembras que presentan ciclos ovulatorios cortos.

2. En cabras expuestas a los machos, 1 o 3 mg de progesterona reducen la proporción de cabras que presentan ciclos ovulatorios cortos.

5. ARTÍCULOS

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Extensive management conditions do not modify the frequency of short ovulatory cycles in progesterone-treated does exposed to sexually active males

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ABSTRACT

Most goats exposed to males in confined conditions have short ovulatory cycles. The frequency of these cycles can be reduced with a progesterone treatment prior to the introduction of males. The objective of this study was to determine whether extensive management conditions modify the frequency of short ovulatory cycles in progesterone-treated does exposed to photostimulated males. One group of does remained in extensive management conditions and grazed daily from 10:00 to 18:00 h; two other groups were confined separately in shaded pens, and fed alfalfa hay. In March, females from the grazing group ($n = 45$; grazing-P4 group) and those from one confined group ($n = 45$; confined-P4 group) were treated with 25 mg of progesterone by intramuscular injections 48 h before joining with photostimulated males ($n = 3$ per group). The other confined group did not receive the progesterone treatment and was exposed to two photostimulated males ($n = 25$; confined-control group). Does were exposed to males for 45 consecutive days. Determination of whether ovulations occurre were made by quantifying plasma progesterone concentrations after introduction of males. The proportion of does that had ovulations at least once was not different between groups ($\geq 98\%$; $P > 0.05$). The proportion of does that had short ovulatory cycles differed between groups ($P < 0.001$), and this proportion was greater in the confined-control group (76%) than in confined-P4 (27%) and grazing-P4 groups (25%; $P < 0.001$). It is concluded that extensive management conditions do not modify the frequency of short ovulatory cycles in progesterone-treated does exposed to the photostimulated males.

1. Introduction

The placement of bucks into a group of seasonal anestrous does can stimulate estrous behavior and induce ovulations within a few days of when the exposure to bucks occurs. This phenomenon of sexual stimulation is known as the "male effect" (Shelton, 1960; Chasles et al., 2016; Zarazaga et al., 2017). First ovulation occurs within 2–8 days following placement of bucks with does, and is not associated with estrous behavior in most does (Ott et al., 1980; Pellicer-Rubio et al., 2016; Ramírez et al., 2017). When mating occurs,

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fertility that is associated with this first ovulation is very low. Does typically have subsequent ovulations 6–9 days later and this second ovulation induced by the buck is generally associated with estrous behavior and followed by an ovulatory cycle of normal duration if pregnancy does not occur as a result of breeding during the period of behavioral estrus. If breeding occurs at this subsequent ovulation fertility rates are what is normally expected (Ott et al., 1980; Pellicer-Rubio et al., 2016; Ramírez et al., 2017). Due to the relatively greater frequency of shorter than typical estrous cycles, there is a longer mating period needed for does (i.e. between 15 and 30 days; Araya et al., 2016). This longer period before mating reduces the period in which females can be induced to initiate ovulations by a photo-stimulated male because the enhanced sexual behaviors in photo-stimulated bucks only lasts for about 2 months (Bedos et al., 2012; Ponce et al., 2014).

With well-nourished does maintained in confined settings, the frequency of short estrous cycles can be reduced by the application of a single injection of 20–25 mg of progesterone 48 h prior to or at the time of buck placement with does (Lassoued et al., 1995; Gonzalez-Bulnes et al., 2006). In addition, when treated with progesterone, does express estrous behaviors that are associated with the time of first ovulation. Furthermore, the synchronization of estrus occurs in a shorter period of time and occurs within 7 days after buck placement with does (Gonzalez-Bulnes et al., 2006; López-Sebastian et al., 2007).In subtropical latitudes, however, most goats are managed in extensive production systems with a diet of only natural vegetation with does having to walk daily 5–8 km and having to graze about 8 h per day if they are to have sufficient dietary intake to maintain their body condition (Ramírez et al., 1991; Lassoued and Rekik, 2005; Delgadillo and Martin, 2015). It, therefore, is likely that the environmental characteristics of the extensive production systems might modify the ovulatory response in does treated with progesterone prior to buck placement with does. Considering the positive effect of progesterone to reduce the proportion of short ovulatory cycles, the present study was conducted to ascertain if does treated with progesterone that were exposed to bucks and managed in an extensive pastoral setting had the same frequency of short ovulatory cycles as progesterone-treated does placed with sexually active males when they were managed in a confined environment where foraging for food did not occur. To this end, there was comparison of the effect of the injection of 25 mg of progesterone in does that had bucks placed with them in a grazing setting to the response of does that had bucks placed with them when they were maintained in a confined setting.

2. Materials and methods

2.1. Ethical note

This study was conducted in accordance with the Official Mexican Norm NOM-062-ZOO-1999 for technical specifications for the production, care and use of laboratory animals (SAGARPA, 2001).

2.2. General conditions of study

The experiment was conducted in the Comarca Lagunera in the state of Coahuila, Mexico (latitude 26°23′N and longitude 104°47′W). The photoperiod in this region varies from 13 h 41 min of the light period at the summer solstice to 10 h 19 min of the light period at the winter solstice. The area is characterized by a dry climate with an average annual rainfall of 266 mm (range: 163 to 504 mm) generally occurring between June and September. Mean annual maximum and minimum temperatures varies from 37 °C between May and August to 6 °C between December and January, respectively (Duarte et al., 2008). Native does and bucks (*Capra hircus*) from Comarca Lagunera region, belonging to the same flock were used for the study. The breed composition of these goats was derived from the Spanish Granadina, Murciana and Malagueña breeds. The goats with this breeding were crossed with Alpine, Saanen, Toggenbourg and Anglo-Nubian breeds in the last 50 years to improve milk and meat production (Delgadillo, 2011). In these does from the Comarca Lagunera, the anestrous period lasts from February to September, and in bucks, the period of relative sexual inactivity (i.e., "rest") lasts from January to June (Delgadillo et al., 1999; Duarte et al., 2008). All does were multiparous and had given birth between August and September, and were milked manually every morning during the study. These does were located in pastoral settings where they grazed only natural vegetation prior the study. At night, does were transferred to open pens. All bucks were separated from does and confined from October until the onset of the study at the Antonio Narro University 20 km from the experimental farm. The bucks were fed with 2 kg of alfalfa hay (17% CP), 200 g of commercial concentrate (14% CP; 2.7 Mcal/kg ME), and had free access to mineral blocks and water.

2.3. Photoperiodic treatment of bucks

The bucks were sexually stimulated by photoperiodic treatment that was previously described (Bedos et al., 2016). Using this protocol, eight sexually experienced bucks that were 3 years-old at the beginning of the study, were subjected to artificial long days (16 h of light/8 h of darkness) from 1 November to 15 January, in a shaded pen (5 × 10 m). After 16 January, bucks were exposed to natural variations of photoperiod until the end of the study. This photoperiodic treatment stimulates testosterone secretion, intensity of odor, and sexual behavior of bucks in March and April, months corresponding to the sexually inactive period of does as a result of seasonal effects on reproduction (Delgadillo et al., 2002; Rivas-Muñoz et al., 2007).

2.4. Females

Anovulation during the anestrous period was determined in each female by trans-rectal ultrasonography performed on 24

February, 6 March, and 13 March using an Aloka SSD-500 device connected to a trans-rectal 7.5 MHz linear probe. Females without *corpora lutea* at the three observational times were considered to be in an anovulatory state (Simões et al., 2007). Immediately after the third ultrasonography, does were randomly assigned to three groups with stratification occurring based on body condition score (2.1 ± 0.1 each group; Walkden-Brown et al., 1997). One group remained in extensive management system and grazed daily from 10:00 to 18:00 h. The other two groups were confined separately in shaded open pens and fed daily with 2 kg of alfalfa hay per animal (confined groups). During milking, each doe received 400 g of commercial concentrate (14% CP; 2.7 Mcal/kg ME).

2.5. "Male effect"

On 13 March, each doe from the grazing group (n = 45; grazing-P4 group) and those from one of the confined groups (n = 45; confined-P4 group) were treated with 25 mg of progesterone by intra-muscular injection in the right side of the neck (Facilgest 25 mg/mL; Syva laboratories). Does from the other confined group did not receive the progesterone treatment (n = 25; confined-control group). On 15 March (day 0), i.e., 48 h after the progesterone treatment, does from the three groups were joined with photo-stimulated, sexually active bucks for 45 consecutive days (n = 3: Grazing-P4 and Confined-P4 groups; n = 2: confined-control group). Bucks and does from the grazing group were maintained in a pastoral setting, and at night were moved into an open shaded pen (20 × 30 m). Animals from both confined groups remained in different open shaded pens (20 × 30 m each). At night, when animals were kept indoors, the distance between the three groups was at least 100 m.

2.6. Measurements

2.6.1. Ovulatory response to the placement of bucks with does

Ovulations were determined by quantifying plasma progesterone concentrations. Jugular blood samples were subsequently obtained from each female on days 0, 3, 6, 9, 12, 15 and 21 after introduction of males into each group of females. Blood samples were collected in 5 mL heparinized tubes and centrifuged at 3000 x g for 30 min at a temperature of 0 °C. The plasma was stored at -20 °C until progesterone concentrations were determined by the immunoenzymatic assay described by Canépa et al. (2008). Sensitivity of assay was 0.25 ng/mL. The intra-assay coefficient of variation was 10.2%. A female was considered to have had an ovulation when the progesterone concentrations were ≥1.0 ng/mL (Chemineau et al., 2006). Pregnancy rates were determined by the presence of embryos or fetuses observed in each female by trans-rectal ultrasonography performed at day 45 after introduction of bucks.

2.7. Definition and statistical analysis of data

The characteristics of the ovulatory response was determined using the methods of Ponce et al. (2015) and Ramírez et al. (2017). These characteristics were: 1) Does that had a short luteal phase followed by a subsequent ovulation: these does had two ovulations and were considered to have had a short ovulatory cycle; (2) Does that had only a short luteal phase which was not followed by any increase in progesterone concentration: these does had one ovulation and returned to the anestrous state without having an ovulatory cycle; and (3) Does that had a normal luteal phase after the first ovulation: these does had an ovulation followed by a normal ovulatory cycle.

The proportions of does that had ovulations, pregnancy rates, and the proportion of does that had short or normal ovulatory cycles were analyzed using the Chi-Square Test. The interval between the introduction of bucks and time of ovulation in does of the three groups was analyzed using a Kruskal-Wallis test. All data were analyzed using the statistical package SYSTAT 13 (2018). Results are expressed as means ± SEM.

3. Results

3.1. Ovulatory response to the "male effect"

The proportions of does that had ovulations at least once were not different between groups as all of them except one doe in the grazing group had an ovulation (P > 0.05; Table 1). The proportion of does that had short ovulatory cycles differed between groups (P < 0.001). The proportion of does having short ovulatory cycles was greater in the confined-control group than in confined-P4 and grazing-P4 groups (P < 0.001; Table 1). As a consequence, does from the confined-control group had a lesser number of normal ovulatory cycles than those from confined-P4 and grazing-P4 groups (P < 0.001; Table 1). The interval between the introduction of bucks and the first ovulation that resulted in short or normal ovulatory cycles did not differ among groups (P > 0.05; Table 1). Furthermore, the pregnancy rates did not differ among the three groups (P > 0.05; Table 1).

4. Discussion

The proportion of does having short ovulatory cycles after exposure to bucks was greater in the confined-control group than in the grazing or confined-P4 groups. In the present study, the proportion of does from the grazing and confined-P4 groups having short ovulatory cycles is similar to those treated with progesterone or a progestagen treatment in confined conditions prior to the placing of bucks with the does (Chemineau, 1985; Gonzalez-Bulnes et al., 2006). Data from the present study suggest that the characteristics of the grazing conditions did not affect the does by modulating the action of progesterone at the hypothalamic-pituitary-gonadal axis to

Table 1

Characteristics of the ovulatory and reproductive responses of seasonally anestrous does placed with sexually active bucks in extensive or confined conditions ($n = 2$ or 3 males per group). Does from the grazing-P4 group grazed natural vegetation from 10:00 to 18:00. Each doe from grazing-P4 and confined-P4 groups were treated with 25 mg of progesterone by intra muscular route in the right side of the neck 48 h before the introduction of males. The confined-control group did not receive the progesterone treatment.

Groups	*n*	Goats with ovulations (%)	Goats with short ovulatory cycles (%)	Goats with normal ovulatory cycles (%)	Pregnancy rates (%)	First ovulation short cycle (days)	First ovulation normal cycle (days)
Grazing-P4	45	44/45 (98)^a	11/44 (25)^a	33/44 (75)^a	40/45 (88)^a	5.2 ± 0.4^a	5.8 ± 0.5^a
Confined-P4	45	45/45 (100)^a	12/44 (27)^a	32/44 (73)^a	38/45 (84)^a	6.0 ± 0.0^a	6.6 ± 0.6^a
Confined-control	25	25/25 (100) ^{a}	19/25 (76) ^{b}	6/25 (24) ^{b}	20/25 (80) ^{a}	5.0 ± 0.5^a	6.0 ± 1.1^a
Chi-Square value		1.569	20.777	20.777	1.043	3.336*	0.879*
P-value		0.456	0.0001	0.0001	0.594	0.189	0.644

^{a,b}Different superscripts within each column indicate differences; $P = 0.0001$; Pearson Chi-Square test.
*Kruskal-Wallis test value.

reduce the short ovulatory cycles in does that had sexually active bucks in the pen or pasture with them. Interestingly, the progesterone treatment reduces not only the proportion of short ovulatory cycles, but also synchronized the timing of ovulations among does in the pastoral setting similar to what occurred with the does in the confined setting. Indeed, most does that had short ovulatory cycles had ovulations within the first 7 days after the bucks were placed in the pen or pasture with them as did does from the confined groups. This result is consistent with that from previous studies where there were progesterone or progestogen-treated does that had bucks placed in the pen with them (Chemineau, 1985; Gonzalez-Bulnes et al., 2006).

In addition, these results indicate that in does that had bucks placed with them in a pastoral setting, the mating period may be reduced to 1 week. This reduction could allow farmers to increase the number of does stimulated by sexually active bucks, because bucks can be used to stimulate several groups of anestrous does. When there is not treatment with progestins, exposure to sexually active bucks results in a stimulation of at least three different groups of anovulatory does when the bucks are placed with does in confined conditions for 15 consecutive days (Bedos et al., 2012). Data from the present study indicate that does treated with progesterone had a 1 week shorter period to initiation of normal ovulatory cycles than does not treated with progesterone which allows for more does to be stimulated by the same bucks. Most does in the grazing group had ovulations at least once during the study, as did does from the confined groups. These findings confirm that when sexually active bucks are used, the system of goat management has only a marginal importance (Rivas-Muñoz et al., 2007; Chasles et al., 2016). Taken together, these findings indicate that progestin treatment reduces short ovulatory cycles in does that had bucks placed with them in pastoral settings, and that when there is placement of sexually active bucks with the does there is induction of ovulations in seasonally anestrous does managed in extensive production systems.

In the present study, pregnancy rates were very good (> 80%) and did not differ in does from the grazing and confined groups. These findings are not consistent with those described for does managed in grazing conditions at subtropical latitudes. In fact, with grazing management, goats feed mainly on the available natural vegetation, and the anestrous season coincides with the dry season, and therefore, with a dramatic reduction of food availability resulting in a reduced body condition of does (Delgadillo and Martin, 2015). In this extensive production system, pregnancy rates and prolificacy in undernourished does exposed to bucks was less than that in well-nourished does (Mellado et al., 1996; Fitz-Rodríguez et al., 2009). Nonetheless, in these undernourished does, a nutritional supplementation during 14 or 28 days after introduction of bucks, improved pregnancy rates (Fitz-Rodríguez et al., 2009). In the present study, does in grazing conditions received a daily nutritional supplementation consisting of 400 g of a commercial concentrate during milking. It is likely that this nutritional supplementation improved the fertility of does maintained in the pastoral setting, which was similar to those in confinement. In does, as in ewes, the nutritional supplementation probably reduced the embryo mortality (Mani et al., 1992; Abecia et al., 2006). As a whole, these data clearly indicate that bucks submitted to long days in confined conditions can be incorporated into a grazing system of management and capacity for fertility will be maintained. This could facilitate the use of sexually active bucks to control the reproductive activity in does in extensive management systems when there is an adequate plane of nutrition.

4.1. Conclusions

In conclusion, the present study indicates that management of does in an extensive production system does not affect the frequency of short ovulatory cycles in progesterone-treated does exposed to the sexually active bucks.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Abecia, J.A., Sosa, C., Forcada, F., Meikle, A., 2006. The effect of undernutrition on the establishment of pregnancy in the ewe. Reprod. Nutr. Dev. 46, 367–378. https://doi.org/10.1051/rnd:2006018.

Araya, J., Bedos, M., Duarte, G., Hernández, H., Keller, M., Chemineau, P., Delgadillo, J.A., 2016. Maintaining bucks over 35 days after a male effect improves pregnancy rate in goats. Anim. Prod. Sci. 57 (10), 2066–2071. https://doi.org/10.1071/AN16194.

Bedos, M., Velázquez, H., Fitz-Rodríguez, G., Flores, J.A., Hernández, H., Duarte, G., Vielma, J., Fernández, I.G., Retana-Márquez, M.S., Muñoz-Gutiérrez, M., Keller, M., Delgadillo, J.A., 2012. Sexually active bucks are able to stimulate three successive groups of females per day with a 4-hour period of contact. Physiol. Behav. 106, 259–263. https://doi.org/10.1016/j.physbeh.2012.02.015.

Bedos, M., Muñoz, A.L., Orihuela, A., Delgadillo, J.A., 2016. The sexual behavior of male goats exposed to long days is as intense as during their breeding season. Appl. Anim. Behav. Sci. 184, 35–40. https://doi.org/10.1016/j.applanim.2016.08.002.

Canépa, S., Lainé, A.L., Bluteau, A., Fagu, C., Flon, C., Monniaux, D., 2008. Validation d'une méthode immunoenzymatique pour le dosage de la progestérone dans le plasma des ovins et des bovins. Cah. Technol. Inra. 64, 19–30.

Chasles, M., Chesneau, D., Moussu, C., Delgadillo, J.A., Chemineau, P., Keller, M., 2016. Sexually active bucks are efficient to stimulate female ovulatory activity during the anestrous season also under temperate latitudes. Anim. Reprod. Sci. 168, 86–91. https://doi.org/10.1016/j.anireprosci.2016.02.030.

Chemineau, P., 1985. Effects of a progestagen on buck-induced short ovarian cycles in the creole meat goat. Anim. Reprod. Sci. 9, 87–94. https://doi.org/10.1016/0378-4320(85)90044-2.

Chemineau, P., Pellicer-Rubio, M.T., Lassoued, N., Khaldi, G., Monniaux, D., 2006. Male-induced short oestrous and ovarian cylces in sheep and goats: a working hypothesis. Reprod. Nutr. Dev. 46, 417–429. https://doi.org/10.1051/rnd:2006022.

Delgadillo, J.A., 2011. Environmental and social cues can be used in combination to develop sustainable breeding techniques for goat reproduction in the subtropics. Animal 5 (1), 74–81. https://doi.org/10.1017/S1751731110001400.

Delgadillo, J.A., Canedo, G.A., Chemineau, P., Guillaume, D., Malpaux, B., 1999. Evidence for an annual reproductive rhythm independent of food availability in male creole goats in subtropical northern Mexico. Theriogenology 52, 727–737. https://doi.org/10.1016/S0093-691X(99)00166-1.

Delgadillo, J.A., Flores, J.A., Véliz, F.G., Hernández, H.F., Duarte, G., Vielma, J., Poindron, P., Chemineau, P., Malpaux, B., 2002. Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days. J. Anim. Sci. 80, 2780–2786. https://doi.org/10.2527/2002.80112780x.

Delgadillo, J.A., Martin, G.B., 2015. Alternative methods for control of reproduction in small ruminants: a focus on the needs of grazing industries. Anim. Front. 5 (1), 57–65. https://doi.org/10.2527/af.2015-0009.

Duarte, G., Flores, J.A., Malpaux, B., Delgadillo, J.A., 2008. Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability. Domest. Anim. Endocrinol. 35, 362–370. https://doi.org/10.1016/j.domaniend.2008.07.005.

Fitz-Rodríguez, G., De Santiago-Miramontes, M.A., Scaramuzzi, R.J., Malpaux, B., Delgadillo, J.A., 2009. Nutritional supplementation improves ovulation and pregnancy rates in female goats managed under natural grazing conditions and exposed to the male effect. Anim. Reprod. Sci. 116, 85–94. https://doi.org/10.1016/j.anireprosci.2009.01.004.

Gonzalez-Bulnes, A., Carrizosa, J.A., Urrutia, B., Lopez-Sebastian, A., 2006. Oestrous behaviour and development of preovulatory follicles in goats induced to ovulate using the male effect with and without progesterone priming. Reprod. Fertil. Dev. 18, 745–750. https://doi.org/10.1071/RD06003.

Lassoued, N., Khaldi, G., Cognié, Y., Chemineau, P., Thimonier, J., 1995. Effet de la progestérone sur le taux d´ovulation et la durée du cycle ovarien induits par effet mâle chez la brebis Barbarine et la chèvre locale tunisienne. Reprod. Nutr. Dev. 35, 415–426. https://doi.org/10.1051/rnd:19950406.

Lassoued, N., Rekik, M., 2005. Variations saisonnières de l´oestrus et de l´ovulation chez la chèvre locale Maure en Tunisie. Rev. Élev. Méd. Vét. Pays Trop. 58 (1–2), 69–73. https://doi.org/10.19182/remvt.9942.

López-Sebastian, A., González-Bulnes, A., Carrizosa, J.A., Urrutia, B., Díaz-Delfa, C., Santiago-Moreno, J., Gómez-Brunet, A., 2007. New estrus synchronization and artificial insemination protocol for goats based on male exposure, progesterone and cloprostenol during the non-breeding season. Theriogenology 68, 1081–1087. https://doi.org/10.1016/j.theriogenology.2007.08.003.

Mani, A.U., McKelvey, W.A.C., Watson, E.D., 1992. The effects of low level of feeding on response to synchronization of estrus, ovulation rate and embryo loss in goats. Theriogenology 38, 1013–1022. https://doi.org/10.1016/0093-691X(92)90115-8.

Mellado, M., Cantú, L., Suárez, J.E., 1996. Effects of body condition, length of breeding period, buck:doe ratio, and month of breeding on kidding rates in goats under extensive conditions in arid zones of Mexico. Small Rumin. Res. 23, 29–35. https://doi.org/10.1016/S0921-4488(96)00896-6.

Ott, R.S., Nelson, D.R., Hixon, J.E., 1980. Effect of presence of the male on initiation of estrous cycle activity of goats. Theriogenology 13 (2), 183–190. https://doi.org/10.1016/0093-691X(80)90127-2.

Pellicer-Rubio, M.T., Boissard, K., Forgerit, Y., Pougnard, J.L., Bonné, J.L., Leboeuf, B., 2016. Evaluation of hormone-free protocols based on the "male effect" for artificial insemination in lactating goats during seasonal anestrus. Theriogenology 85, 960–969. https://doi.org/10.1016/j.theriogenology.2015.11.005.

Ponce, J.L., Velázquez, H., Duarte, G., Bedos, M., Hernández, H., Keller, M., Chemineau, P., Delgadillo, J.A., 2014. Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of males goats to stimulate ovulatory activity in seasonally anoulatory females. Domest. Anim. Endocrinol. 48, 119–125. https://doi.org/10.1016/jdomaniend.2014.03.002.

Ponce, J.L., Hernández, H., Flores, J.A., Keller, M., Chemineau, P., Delgadillo, J.A., 2015. One day of contact with photostimulated bucks is sufficient to induce ovulation in seasonally anestrous goats. Theriogenology 84, 880–886. https://doi.org/10.1016/j.theriogenology.2015.05.019.

Ramirez, R.G., Loyo, A., Mora, R., Sánchez, E.M., Chaire, A., 1991. Forage intake and nutrition of range goats in a shrubland in northeastern Mexico. J. Anim. Sci. 69, 879–885. https://doi.org/10.2527/1991.693879x.

Ramírez, S., Bedos, M., Chasles, M., Hernández, H., Flores, J.A., Vielma, J., Duarte, G., Retana-Márquez, M.S., Keller, M., Chemineau, P., Delgadillo, J.A., 2017. Fifteen minutes of daily contact with sexually active male induces ovulation but delays its timing in seasonally anestrous goats. Theriogenology 87, 148–153. https://doi.org/10.1016/j.theriogenology.2016.08.019.

Rivas-Muñoz, R., Fitz-Rodríguez, G., Poindron, P., Malpaux, B., Delgadillo, J.A., 2007. Stimulation of estrous behavior in grazing female goats by continuous or discontinuous exposure to males. J. Anim. Sci. 85, 1257–1263. https://doi.org/10.2527/jas.2006-115.

Secretaría De Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2001. 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. June 18th 2001. .

Simões, J., Almeida, J.C., Baril, G., Azevedo, J., Fontes, P., Mascarenhas, R., 2007. Assessment of luteal function by ultrasonographic appearance and measurement of corpora lutea in goats. Anim. Reprod. Sci. 97, 36–46. https://doi.org/10.1016/j.anireprosci.2006.01.006.

Shelton, M., 1960. Influence of the presence of a male goat on the initiation of estrous cycling and ovulation of Angora does. J. Anim. Sci. 19 (2), 368–375. https://doi.org/10.2527/jas1960.192368x.

Systat Version 13.2. 2018, Systat Software, Inc. San Jose, CA 95131 USA.

Walkden-Brown, S.W., Restall, B.J., Scaramuzzi, R.J., Martin, G.B., Blackberry, M.A., 1997. 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, 239–252. https://doi.org/10.1016/S0921-4488(97)00017-5.

Zarazaga, L.A., Gatica, M.C., Hernández, H., Gallego-Calvo, L., Delgadillo, J.A., Guzmán, J.L., 2017. The isolation of females from males to promote a later male effect is unnecessary if the bucks used are sexually active. Theriogenology 95, 42–47. https://doi.org/10.1016/j.theriogenology.2017.02.023.

Manuscript Details

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Title	Progesterone doses of 1, 3 or 5 mg do not prevent short ovulatory cycles in goats exposed to photo-stimulated bucks
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Abstract

In seasonal anestrous goats exposed to males, literature reports that 5 mg of progesterone prevents short ovulatory cycles. The aim of this study was to determine whether lower doses than 5 mg of progesterone reduce the frequency of these short ovulatory cycles in seasonal anestrous goats exposed to sexually active bucks. Females from the control group were given an im dose of 2 mL olive oil (n = 9). Females from the experimental groups were given im 1 mg (n = 15), 3 mg (n = 16), 5 mg (n = 15) or 25 mg (n = 16) of progesterone diluted in 2 mL olive oil, 48 h prior exposition to bucks (n = 1 per group). The proportion of goats that ovulated was high (≥87%), and was not different between groups (P>0.05). In contrast, the proportion of goats that displayed short ovulatory cycles differed between groups (P<0.05), and these proportions were higher in control goats (78%), and in those treated with 1 mg (85%), 3 mg (50%), or 5 mg (71%) than in those treated with 25 mg (12%; P<0.05). Finally, fertility (≥40%) and prolificacy (≥1.4) did not differ between groups (P>0.05). We conclude that 1, 3 or 5 mg of progesterone, on the contrary of 25 mg, do not reduce the frequency of short ovulatory cycles in seasonal anestrous goats exposed to the male effect.

Keywords	Caprine, buck effect, fertility, prolificacy
Taxonomy	Animal Breeding, Goat

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Dear Dr. F. Gandolfi
Editor in Chief
Theriogenology

October 9, 2018

Please find enclosed our manuscript “Progesterone doses of 1, 3 or 5 mg do not prevent short ovulatory cycles in goats exposed to photo-stimulated bucks”.

Most females goats exposed to males during the seasonal anestrous display short ovulatory cycles. In goats, the frequency of these short ovulatory cycles can be reduced by the application of a single injection of 25 mg of progesterone 48 h prior or at male introduction. In addition, literature indicates that 5 mg of progesterone also prevent these short ovulatory cycles. Nonetheless, we do not know whether lower doses than 5 mg of progesterone reduce the frequency of these short ovulatory cycles in seasonal anestrous goats exposed to sexually active bucks. Therefore, we used progesterone doses of 1, 3, 5, or 25 mg in goats exposed to the male effect.

Our results showed that most of goats ovulated (≥87%), and there were not differences between groups (P>0.05). in contrast, the proportion of goats that dsiplayed short ovulatory cycles differed between groups, and these proportions were higher in goats treated with 1 mg (85%), 3 mg (50%), or 5 mg (71%) than in those treated with 25 mg (12%; P<0.05). Fertility (≥40%) and prolificacy (≥1.4) did not differ between groups (P>0.05).

Therefore, we consider that these results have important practical applications, because they show that progesterone doses of 1, 3 or 5 mg do not prevent the short ovulatory cycles. For reduce this frequency in goats exposed to the male effect, we need to use 25 mg of progesterone.

Most goats exposed to males display short ovulatory cycles

These short ovulatory cycles are reduced in goats receiving 20 mg of progesterone

Progesterone doses of 1, 3 or 5 mg do not prevent these short ovulatory cycles

Progesterone doses of 1, 3 or 5 mg do not prevent short ovulatory cycles in goats exposed to photo-stimulated bucks

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Abstract

In seasonal anestrous goats exposed to males, literature reports that 5 mg of progesterone prevent short ovulatory cycles. The aim of this study was to determine whether lower doses than 5 mg of progesterone reduce the frequency of these short ovulatory cycles in seasonal anestrous goats exposed to sexually active bucks. Females from the control group were given an im dose of 2 mL olive oil (n = 9). Females from the experimental groups were given im 1 mg (n = 15), 3 mg (n = 16), 5 mg (n = 15) or 25 mg (n = 16) of progesterone diluted in 2 mL olive oil, 48 h prior exposition to bucks (n = 1 per group). The proportion of goats that ovulated was high (≥87%), and was not different between groups (P>0.05). In contrast, the proportion of goats that displayed short ovulatory cycles differed between groups (P<0.05), and these proportions were higher in control goats (78%), and in those treated with 1 mg (85%), 3 mg (50%), or 5 mg (71%) than in those treated with 25 mg (12%; P<0.05). Finally, fertility (≥40%) and prolificacy (≥1.4) did not differ between groups (P>0.05). We conclude that 1, 3 or 5 mg of progesterone, on the contrary of 25 mg, do not reduce the frequency of short ovulatory cycles in seasonal anestrous goats exposed to the male effect.

Keywords: Caprine, buck effect, fertility, prolificacy

1. Introduction

In goats and ewes that display seasonal anestrous, the introduction of bucks or rams stimulates estrous behavior and ovulatory activity in the following days. This technique of stimulation is called the male effect [1,2,3]. In goats, most females ovulate within 2-8 days after male introduction, however, a high proportion of these ovulations are not associated with estrous behavior [4,5,6]. Thereafter, goats ovulate again 6-9 days later and this second ovulation is generally associated with estrous behavior and followed by an ovulatory cycle of normal duration. This second ovulation is fertile in most females [4,5,6]. Due to the high occurrence of short estrous cycles in goats exposed to males, the mating period must be prolonged by about 30 days to give opportunity to females to become pregnant [7].

In goats and ewes, the application of a single dose of 20 or 25 mg of progesterone before or at the introduction of males reduces considerably or prevents the occurrence of short ovulatory cycles [8,9,10,11]. In ewes, the reduction of these short ovulatory cycles depends on progesterone doses. About 46-93% of ewes not treated with progesterone displays short ovulatory cycles when exposed to males [8,2]. By contrast, this frequency is reduced to 15%, 12% and 0% in females treated respectively with 5, 10 or 20 mg of progesterone [8]. In goats, more than 80% of females untreated with progesterone display short ovulatory cycles when exposed to males [12,5,6]. Interestingly, in goats exposed to males, 5.2 mg of fluorogestone acetate or 5 mg of progesterone prevents short ovulatory cycles [12,13]. Considering that in goats 5 mg of progesterone prevents short ovulatory cycles, we tested whether the dose of progesterone could be further reduced to 1 or 3 mg of progesterone. These two experimental groups were compared with females that received 5 or 25 mg of progesterone and a control group receiving the vehicle injection (olive oil).

2. Materials and methods

2.1. Ethical note

The procedures used in this experiment were in accordance to the Official Mexican Norm NOM-062-ZOO-1999, Technical specifications for the production, care and use of laboratory animals [14].

2.2. Location and general conditions of study

The study was performed in the Comarca Lagunera, state of Coahuila, Mexico (latitude 26°23´N; longitude 104°47´W), during the non-breeding season using local female and male goats (*Capra hircus*). In females, the seasonal anestrous lasts from March to August; while in bucks the sexual rest lasts from January to May [15,16]. All females used in this study were multiparous and had given birth between August and October. They were milked manually once daily during the study. Throughout the experiment, females and males were kept indoors and fed with 2 kg of alfalfa hay (17% crude protein) and 200 g of commercial concentrate per day (2.7 Mcal/kg ME). They had free access to water and mineral licks.

2.3. Photoperiodic treatment of bucks

Five bucks were housed in a 5 x 10 m shaded open pen, and exposed to artificial long days (16 h light/8 h darkness) from November 1st to January 15th combining natural and artificial light. From January 16th until the end of the study, males were exposed to the natural variations of photoperiod. The characteristics of the pens and photoperiodic treatment were previously described [17]. Briefly, artificial daylight was given by 12 lamps of 65 W of energy each, placed at 2.6 m of the ground and distributed along the pen. These lamps provided an intensity light of at least 300 lux measured laterally to the eyes of the animals. The switching on and off of lamps was regulated by an electric timer which turned on from 06:00 to 08:00 h and from 18:00 to 22:00 h. About 1.5 months after the end of the extra-light treatment testosterone secretion is stimulated and therefore, improves sexual behavior of bucks from late February to late April, months that correspond to normal sexual rest [18,19,20].

2.4. Selection of females

Goats were isolated from bucks from September until the onset of the study. Anovulatory state of goats was determined by three transrectal ultrasonography performed on February 26th, March 7th, and March 17th, using an Aloka SSD-500 device connected to a transrectal 7.5 MHz linear probe. Females that did not have any corpus luteum at the time of the three ultrasonography were considered as being in seasonal anovulation [21].

2.5. Application of progesterone and the male effect

On March 20th females were divided into five groups balanced by body condition score (1.8 ± 0.1 each group) [22]. On March 22th, the control group was given an im dose of 2 mL of olive oil (n = 9). The other groups received an im injection of 1 mg (n = 15), 3 mg (n = 16), 5 mg (n= 15) or 25 mg (n = 15) of progesterone 48 h before male introduction in each group. Each dose of progesterone was diluted in 2 mL olive oil before administration [23]. On March 24th (Day 0), females of each group were put in contact with photostimulated bucks (n = 1 per group), which were daily switched between groups to avoid an individual effect. Males remained with goats during 21 consecutive days. The distance between groups was more than 100 m to avoid any interference between groups.

2.6. Measurements

Ovulatory activity was appreciated by measuring plasma progesterone concentrations. Five mL of blood samples were collected by jugular venipuncture from each female in tubes containing 30 µL of heparin. Females were sampled daily from 4 days before the introduction of males to 21 days after this introduction. Blood samples were immediately centrifuged at 3500 X g for 30 minutes and the plasma obtained was stored at -20°C until progesterone concentrations were determined by immunoenzymatic assay described by Canépa et al. [24]. Sensitivity was 0.25 ng/mL. Coefficients of variation intra-assay and inter-assay were 6.9% and 8.7%, respectively. Females with progesterone concentrations >1 ng/mL were considered to have ovulated [1]. Fertility and prolificacy were determined at parturition [17].

2.7. Statistical analysis

The proportions of females that ovulated, goats that displayed short ovulatory cycle, and fertility rates were analyzed using the Chi-Square Test. When there was a statistical difference, the comparisons between groups were made using the Fisher Exact Test. Prolificacy was analyzed using a Kruskal-Wallis Test. All data were analyzed using the statistical package SYSTAT 13 [25]. Results are expressed as mean ± standard error of the mean (SEM).

3. Results

The proportion of goats that ovulated at least once did not differ between groups ($P>0.05$; Table 1). By contrast, the proportion of goats that exhibit short ovulatory cycle was different between groups ($P<0.05$). Indeed, the proportion of goats displaying short ovulatory cycles was lower in goats that received 25 mg of progesterone compared with the other four groups ($P<0.05$; Figure 1; Table 1). On the contrary, the proportion of goats displaying short ovulatory cycles did not differ between females from control group and those receiving 1, 3, or 5 mg of progesterone. Consequently, goats treated with 25 mg of progesterone displayed more normal ovulatory cycles than those from the rest of the experimental groups ($P<0.05$; Figure 1; Table 1). Fertility and prolificacy did not differ between groups ($P>0.05$; Table 1).

4. Discussion

The proportion of goats displaying short ovulatory cycles after exposure to bucks was dramatically reduced in goats treated with 25 mg of progesterone.

By contrast, lower doses such as 1, 3 or 5 mg of progesterone failed to reduce this frequency, and therefore, the proportions of the short ovulatory cycles were higher than in females treated with 25 mg of progesterone. In this later group, however, there are still two females experiencing a short ovulatory cycle, which probably indicates that 25 mg is close or below the minimal efficient dose reported in the literature to completely suppresses these short cycles. Interestingly, progesterone treatments did not modify the proportions of females that ovulated, fertility or prolificacy, and there was no difference between groups in that regard. As a whole, our results do not support our initial hypothesis, and indicate that in the conditions of the current study, doses of 5 mg of progesterone or lower do not reduce the frequency of short ovulatory cycles in goats exposed to males.

In our study, 25 mg of progesterone decreases the frequency of short ovulatory cycles, whereas lower doses did not so. Our findings agree with those reported in goats and ewes exposed to males, in which a treatment with 20 or 25 mg of progesterone dramatically decrease or prevent short ovulatory cycles [26,10,11]. In goats and ewes, the efficacy of lower doses than 20 or 25 mg of progesterone to prevent the short ovulatory cycles varied between species. Thus, in goats exposed to males, 5 mg of progesterone was reported to prevent the short ovulatory cycles [13]. In contrast, about 15% of ewes treated with 5 mg of progesterone displayed short ovulatory cycles when exposed to rams [26,8]. In our study, the proportion of females that displayed short ovulatory cycles was high (>50%) and did not differ among those treated with 0, 1, 3, or 5 mg of progesterone. Contrary to that was described in goats [13], in our study 5 mg of progesterone failed to prevent short ovulatory cycles. The causes of this difference are not clear. Nonetheless, the fact that in our study 5 mg of progesterone failed to prevent or reduce short ovulatory cycles can be explained by two nonexclusive hypotheses. Firstly, in ewes it seems that a plasma progesterone concentration of ≥ 0.5 ng/mL is necessary to prevent the short ovulatory cycles. Therefore, an im dose of 25 mg of progesterone diluted in 2 mL of olive oil produce a maximum plasma concentration of about 2.5 ng/mL, 3 h after injection, reducing the frequency of short ovulatory cycles [8,23]. In our study, we were not able to determine the maximum plasma progesterone concentrations produced by the different administered doses, because females were sampled 24 h after progesterone treatment. However, we can speculate that in our study, the plasma progesterone concentrations induced by the low doses of progesterone (1, 3, 5 mg) were lower than 2.5 ng/mL, and as a consequence, short ovulatory cycles were not prevented. Secondly, in ewes, the efficiency of progesterone treatment to prevent the short ovulatory cycles depends also on a minimal duration above the effective concentration (30h ≥ 0.5 ng/mL) [23]. In our study, we were not able to determine the pharmacokinetic of exogenous progesterone because females were sampled every 24 h. Nonetheless, we can speculate that the low doses of progesterone we used, did not allow to maintain progesterone levels ≥ 0.5 ng/mL for a sufficient duration to prevent the short ovulatory cycles. Taken together, our data indicates that a single administration of progesterone doses greater than 5 mg, and probably close to 25 mg or even higher, are necessary to avoid the short ovulatory cycles.

In our study, the photo-stimulated males were able to trigger the ovulatory and reproductive responses in most goats, and the proportions of goats that ovulated, fertility at kidding and prolificacy did not differ among groups. These findings agree with others reports by our group in progesterone-untreated goats exposed to photo-stimulated males [27,28,29]. Then, our results indicate that the ovulatory and reproductive responses were not modified by progesterone treatments. Therefore, it is likely the high ovulatory and reproductive responses were due to the intense sexual behavior displayed by the photo-stimulated males. In fact, several studies showed that photo-stimulated males are able to stimulate LH secretion and ovulations in seasonal anestrous goats [30,31,20].

We concluded that doses of 1, 3 or 5 mg of progesterone on the contrary of 25 mg, do not prevent the short ovulatory cycles in goats exposed to the photo-stimulated bucks.

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References

1. Chemineau P, Pellicer-Rubio MT, Lassoued N, Khaldi G, Monniaux D. Male-induced short oestrous and ovarian cylces in sheep and goats: a working hypothesis. Reprod Nutr Dev 2006;46:417-429. http://doi:10.1051/rnd:2006022

2. Chanvallon A, Sagot L, Pottier E, Debus N, Francois 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(10):1594-1604. http://doi:10.1017/S1751731111000668

3. Zarazaga LA, Gatica MC, Hernández H, Gallego-Calvo L, Delgadillo JA, Guzmán JL. The isolation of females from males to promote a later male effect is unnecessary if the bucks used are sexually active. Theriogenology 2017;95:42-47. http://dx.doi.org/10.1016/j.theriogenology.2017.02.023

4. Ott RS, Nelson DR, Hixon JE. Effect of presence of the male on initiation of estrous cycle activity of goats. Theriogenology 1980;13(2):183-190. https://doi.org/10.1016/0093-691X(80)90127-2

5. Pellicer-Rubio MT, Boissard K, Forgerit Y, Pougnard JL, Bonné JL, Leboeuf B. Evaluation of hormone-free protocols based on the “male effect” for artificial insemination in lactating goats during seasonal anestrus.

Theriogenology 2016;85:960-969. http://dx.doi.org/10.1016/j.theriogenology.2015.11.005

6. Ramírez S, Bedos M, Chasles M, Hernández H, Flores JA, Vielma J, Duarte G, Retana-Márquez MS, Keller M, Chemineau P, Delgadillo JA. Fifteen minutes of daily contact with sexually active male induces ovulation but delays its timing in seasonally anestrous goats. Theriogenology 2017;87:148-153. http://dx.doi.org/10.1016/j.theriogenology.2016.08.019

7. Araya J, Bedos M, Duarte G, Hernández H, Keller M, Chemineau P, Delgadillo JA. Maintaining bucks over 35 days after a male effect improves pregnancy rate in goats. Anim Prod Sci 2016;57(10):2066-2071. http://dx.doi.org/10.1071/AN16194

8. Oldham CM, Pearce DT, Gray SJ. Progesterone priming and age of ewe affect the life-span of corpora lutea induced in the seasonally anovulatory Merino ewe by the "ram effect". J Reprod Fert 1985;75:29-33.

9. Pearce DT, Martin GB, Oldham CM. Corpora lutea with a short life-span induced by rams in seasonally anovulatory ewes are prevented by progesterone delaying the preovulatory surge of LH. J Reprod Fert 1985;75:79-84.

10. Lassoued N, Khaldi G, Cognié Y, Chemineau P, Thimonier J. Effet de la progestéronesur le tauxd´ovulation et la durée du cycle ovarieninduits par effet mâle chez la brebisBarbarine et la chèvre locale tunisienne. Reprod Nutr Dev 1995;35:415-426.

11. Gonzalez-Bulnes A, Carrizosa JA, Urrutia B, Lopez-Sebastian A. Oestrous behaviour and development of preovulatory follicles in goats induced to ovulate using the male effect with and without progesterone priming. Reprod Fertil Dev 2006;18:745-750. https://doi.org/10.1071/RD06003

12. Chemineau P. Effects of a progestagen on buck-induced short ovarian cycles in the creole meat goat. Anim Reprod Sci 1985;9:87-94. https://doi.org/10.1016/0378-4320(85)90044-2

13. Véliz FG, Meza-Herrera CA, De Santiago-Miramontes MA, Arellano-Rodríguez G, Leyva C, Rivas-Muñoz R, Mellado M. Effect of parity and progesterone priming on induction of reproductive function in Saanen goats by buck exposure. Livest Sci 2009;125:261-265. http://doi:10.1016/j.livsci.2009.05.008

14. Secretaría De Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2001. 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. June 18th, 2001.

15. Delgadillo JA, Canedo GA, Chemineau P, Guillaume D, Malpaux B. Evidence for an annual reproductive rhythm independent of food availability in male creole goats in subtropical northern Mexico. Theriogenology 1999;52:727-737. https://doi.org/10.1016/S0093-691X(99)00166-1

16. 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;35:362-370. http://doi:10.1016/j.domaniend.2008.07.005

17. Bedos M, Muñoz AL, Orihuela A, Delgadillo JA. The sexual behavior of male goats exposed to long days is as intense as during their breeding season. Appl Anim Behav Sci 2016;184:35–40. http://dx.doi.org/10.1016/j.applanim.2016.08.002

18. Delgadillo JA, Flores JA, Véliz FG, Hernández HF, Duarte G, Vielma J, Poindron P, Chemineau P, Malpaux B. Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days. J Anim Sci 2002;80:2780-2786. https://doi.org/10.2527/2002.80112780x

19. Rivas-Muñoz R, Fitz-Rodríguez G, Poindron P, Malpaux B, Delgadillo JA. Stimulation of estrous behavior in grazing female goats by continuous or discontinuous exposure to males. J Anim Sci 2007;85:1257-1263. http://doi:10.2527/jas.2006-115

20. Ponce JL, Velázquez H, Duarte G, Bedos M, Hernández H, Keller M, Chemineau P, Delgadillo JA. Reducing exposure to long days from 75 days 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;48:119-125. http://dx.doi.org/10.1016/j.domaniend.2014.03.002

21. Delgadillo JA, Ungerfeld R, Flores JA, Hernández H, Fitz-Rodríguez G. The ovularory response of anestrous goats exposed to the male effect in the subtropics is unrelated to their follicular diameter at male exposure. Reprod Dom Anim 2011;46:687-691. http://doi: 10.1111/j.1439-0531.2010.01730.x

22. Walkden-Brown SW, Martin GB, Restall BJ. Role of male-female interaction in regulating reproduction in sheep and goats. J Reprod Fertil Suppl 1999;52:243-257.

23. Contreras-Solis I, Gomez-Brunet A, Encinas T, Gonzalez-Bulnes A, Santiago-Moreno J, Lopez-Sebastian A. Influence of vehicle on kinetics of exogenous progesterone administered either by subcutaneous and intramuscular routes to sheep. Res Vet Sci 2008;85:162-165. http://doi:10.1016/j.rvsc.2007.09.011

24. Canépa S, Lainé AL, Bluteau A, Fagu C, Flon C, Monniaux D. Validation d'une méthodeimmunoenzymatique pour le dosage de la progestéronedans le plasma des ovins et des bovins. Cah Techn Inra 2008;64:19-30.

25. Systat Version 13.2. 2018, Systat Software, Inc. San Jose, CA 95131 USA.

26. Cognié Y, Gray SJ, Lindsay DR, Oldham CM, Pearce DT, Signoret JP. A new approach to controlled breeding in sheep using the ram effect. Proc Aust Soc Anim Prod 1982;14:519-522. http://livestocklibrary.com.au/handle/1234/7284

27. Bedos M, Velázquez H, Fitz-Rodríguez G, Flores JA, Hernández H, Duarte G, Vielma J, Fernández IG, Retana-Márquez MS, Muñoz-Gutiérrez M, Keller M, Delgadillo JA. Sexually active bucks are able to stimulate three successive groups of females per day with a 4-hour period of contact. Physiol Behav 2012;106:259–263. http://doi:10.1016/j.physbeh.2012.02.015

28. 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;146:21-26. http://dx.doi.org/10.1016/j.anireprosci.2014.02.002

29. Chasles M, Chesneau D, Moussu C, Delgadillo JA, Chemineau P, Keller M. Sexually active bucks are efficient to stimulate female ovulatory activity during the anestrous season also under temperate latitudes. Anim Reprod Sci 2016;168:86-91. http://dx.doi.org/doi:10.1016/j.anireprosci.2016.02.030

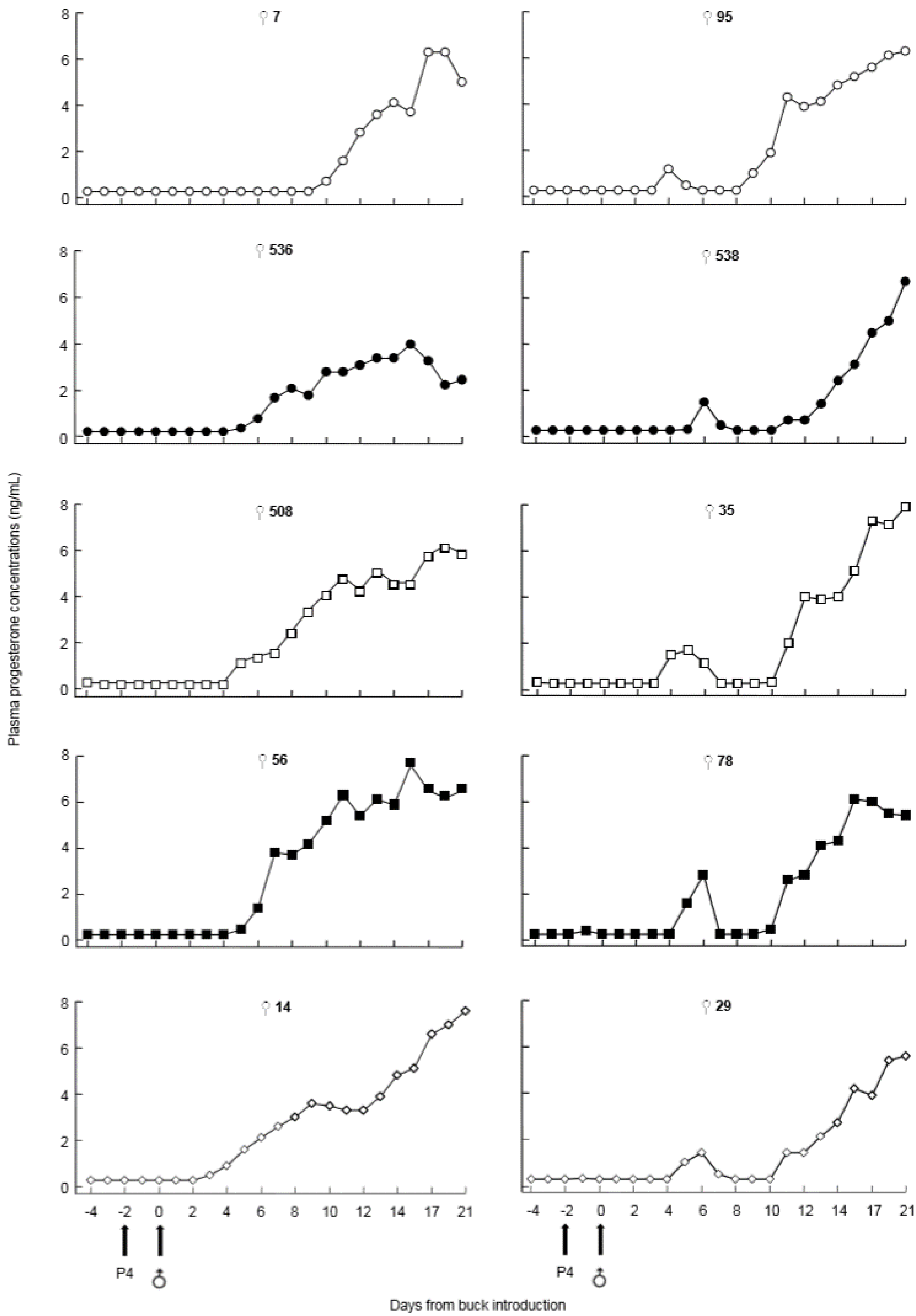
30. Vielma J, Chemineau P, Poindron P, Malpaux B, Delgadillo JA. Male sexual behavior contributes to the maintenance of high LH pulsatility in anestrous female goats. Horm Behav 2009,56:444-449. http://doi:10.1016/j.yhbeh.2009.07.015

31. 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;48:93-99.http://doi.10.1016/j.domaniend.2014.02.003.

Figure legends

Figure 1. Examples of individual patterns of plasma progesterone concentrations in goats exposed to bucks 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. Bucks were introduced on March 24th. Goats from the control group were given im dose of 2 mL olive oil (○); females from the experimental groups were given im doses of 1 mg (●), 3 mg (□), 5 mg (■) or 25 mg (♦) of progesterone diluted in 2 mL olive oil 48 h prior exposition to bucks (n = 1 per group). ↑ Indicates the timing of progesterone treatment (P4) and of the introduction of males (♂).



♀ 7
♀ 95
♀ 536
♀ 538
♀ 508
♀ 35
♀ 56
♀ 78
♀ 14
♀ 29
Plasma progesterone concentrations (ng/mL)
0
2
4
6
8
-4
-2
0
2
4
6
8
10
12
14
17
21
P4
♂
Days from buck introduction

Table 1. Characteristics of the ovulatory response and reproductive parameters of anestrous goats exposed to sexually active males. Goats were given 1, 3, 5 or 25 mg of progesterone diluted in 2 mL olive oil 48 h before buck introduction. Control group was given an im dose of 2 mL olive oil.

Groups	n	Goats that ovulated (%)	Goats with short ovulatory cycles (%)	Fertility (%)	Prolificacy (%)
Control group	9	9/9 (100)[a]	7/9 (78)[a]	7/9 (78)[a]	1.4 ± 0.2^{a}
1 mg	15	13/15 (87)[a]	11/13 (85)[a]	6/15 (40)[a]	1.5 ± 0.2^{a}
3 mg	16	16/16 (100)[a]	8/16 (50)[a]	11/16 (69)[a]	1.5 ± 0.2^{a}
5 mg	15	14/15 (93)[a]	10/14 (71)[a]	9/15 (60)[a]	1.4 ± 0.2^{a}
25 mg	16	16/16 (100)[a]	2/16 (12)[b]	11/16 (69)[a]	1.4 ± 0.2^{a}

[a,b]Different superscripts within each column indicate significant difference (<0.05)

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The authors confirm the following statements:

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J.A. Delgadillo	

6. DISCUSIÓN GENERAL

Los resultados del estudio 1 muestran que, en las cabras expuestas al efecto macho en un sistema de producción extensivo, 25 mg de progesterona reducen la proporción de hembras que presentan ciclos ovulatorios cortos, de la misma manera que en hembras mantenidas en condiciones intensivas (Lassoued *et al.,* 1995; Gonzalez-Bulnes *et al.,* 2006). En efecto, en el presente estudio menos del 30% de las cabras mantenidas en los sistemas extensivo o intensivo presentaron ciclos ovulatorios cortos, y la mayoría de las hembras ovularon en los primeros 7 días de contacto con los machos. En cambio, más del 70% de las cabras mantenidas en el sistema intensivo y no tratadas con progesterona, presentaron ciclos ovulatorios cortos. Estos datos sugieren que, en cabras mantenidas en un sistema de producción extensivo, el periodo de empadre puede reducirse a una semana, dando la oportunidad de incrementar el número de hembras estimuladas por un macho sexualmente activo, sin reducir la fertilidad de las hembras (Bedos *et al.,* 2012). Sería interesante determinar la fertilidad de las cabras inseminadas artificialmente al tratarlas con progesterona y exponerlas en un sistema de producción extensivo al efecto macho.

Las proporciones de cabras que ovularon (≥98%), y las que quedaron gestantes (>80%) no difirieron entre las hembras mantenidas en los sistemas de producción extensivo o intensivo, y coinciden con lo reportado anteriormente en cabras mantenidas en sistemas intensivos (Lassoued *et al.,* 1995; González-Bulnes *et al.,* 2006). Estos datos indican que las características propias del sistema de producción extensivo no tuvieron efecto sobre la respuesta al efecto macho. Asimismo, los datos de este estudio indican que los machos sometidos a un tratamiento de días largos artificiales en un sistema de producción intensivo, son eficaces para estimular la actividad sexual y reproductiva de las cabras cuando son trasladados a un sistema de producción extensivo.

Los resultados del estudio 2 muestran que 1, 3 o 5 mg de progesterona no reducen la proporción de cabras que presentan ciclos ovulatorios cortos al ser expuestas a los machos fotoestimulados. En efecto, las proporciones de cabras que presentaron ciclos ovulatorios cortos en estos tres grupos fueron elevadas (≥50%), y no difirieron del grupo control que no se trató con progesterona (78%). En cambio, en el grupo tratado con 25 mg de progesterona, la proporción de cabras que presentaron ciclos ovulatorios cortos fue significativamente inferior (12%). Estos resultados, que difieren de los reportados previamente (Véliz *et al.,* 2009), sugieren que 25 mg de progesterona es, muy probablemente, la dosis mínima para reducir los ciclos ovulatorios cortos en cabras al ser expuestas a los machos. Esta hipótesis es apoyada por los resultados del experimento 1, y por los reportados en otros estudios en la especie caprina (Lassoued *et al.,* 1995; González-Bulnes *et al.,* 2006). Es imporante señalar que la respuesta reproductiva de las cabras no fue diferente entre los grupos, lo que sugiere que 25 mg de progesterona no sólo reduce la presentación de los ciclos cortos, sino que además no disminuye la fertilidad y prolificidad de las hembras expuestas al efecto macho.

Se concluye que i) el sistema de producción extensivo no modifica la proporción de cabras que presentan ciclos ovulatorios cortos en hembras tratadas con 25 mg de progesterona y expuestas al efecto macho, y que ii) La aplicación de 1, 3 o 5 mg de progesterona no son suficientes para reducir la proporción de cabras que presentan ciclos ovulatorios cortos al exponerlas al efecto macho.

7. REFERENCIAS

ABECIA, J. A., P. CHEMINEAU, M. KELLER, J. A. DELGADILLO. Extended day length in late winter/early spring, with a return to natural day length of shorter duration, increased plasma testosterone and sexual performance in rams with or without melatonin implants. *Reproduction in Domestic Animals.* 2017, 52 (5), 851-856.

ARAYA, J., M. BEDOS, G. DUARTE, H. HERNÁNDEZ, M. KELLER, P. CHEMINEAU, J. A. DELGADILLO. Maintaining bucks over 35 days after a male effect improves pregnancy rate in goats. *Animal Production Science.* 2016, 57 (10), 2066-2071.

BARIL, G., P. CHEMINEAU, Y. COGNIÉ, Y. GUÉRIN, B. LEBOEUF, P. ORGEUR, J. C. VALLET. *Manuel de formation pour l´isémination artificielle chez les ovins et les caprins.* Étude FAO Production Et Santé Animales 83. Rome, Italie. FAO. 1993a. 231 p. ISBN 92-5-202808-0

BARIL, G., B. LEBOEUF, J. SAUMANDE. Synchronization of estrus in goats: the relationship between time of occurrence of estrus and fertility following artificial insemination. *Theriogenology.* 1993b, 40, 621-628.

BEDOS, M., H. VELÁZQUEZ, G. FITZ-RODRÍGUEZ, J. A. FLORES, H. HERNÁNDEZ, G. DUARTE, J. VIELMA, I. G. FERNÁNDEZ, M. S. RETANA-MÁRQUEZ, M. MUÑOZ-GUTIÉRREZ, M. KELLER, J. A. DELGADILLO. Sexually active bucks are able to stimulate three successive groups of females per day with a 4-hour period of contact. *Physiology and Behavior.* 2012, 106 (2), 259-263.

BEDOS, M., A. L. MUÑOZ, A. ORIHUELA, J. A. DELGADILLO. The sexual behavior of male goats exposed to long days is as intense as during their breeding season. *Applied Animal Behaviour Science.* 2016, 184, 35–40.

CHEMINEAU, P. Effects of a progestagen on buck-induced short ovarian cycles in the creole meat goat. *Animal Reproduction Science.* 1985, 9, 87-94.

CHEMINEAU, P. Possibilities for using bucks to stimulate ovarian and oestrous cycles in anovulatory goats - a review. *Livestock Production Science.* 1987, 17, 135-147.

CHEMINEAU, P., B. MALPAUX, J. A. DELGADILLO, Y. GUÉRIN, J. P. RAVAULT, J. THIMONIER, J. PELLETIER. Control of sheep and goat reproduction: use of light and melatonin. *Animal Reproduction Science.* 1992, 30, 157-184.

CHEMINEAU, P., M. T. PELLICER-RUBIO, N. LASSOUED, G. KHALDI, D. MONNIAUX. Male-induced short oestrous and ovarian cycles in sheep and goats: a working hypothesis. *Reproduction Nutrition Development.* 2006, 46, 417-429.

CHEMINEAU, P., B. MALPAUX, J. P. BRILLARD, A. FOSTIER. Seasonality of reproduction and production in farm fishes, birds and mammals. *Animal.* 2007, 1, 419-432.

CHEMINEAU, P., D. GUILLAUME, M. MIGAUD, J. C. THIÉRY, M. T. PELLICER-RUBIO, B. MALPAUX. Seasonality of reproduction in mammals: intimate regulatory mechanisms and practical implications. *Reproduction in Domestic Animals.* 2008, 43 (Suppl. 2), 40-47.

DELGADILLO, J. A. Environmental and social cues can be used in combination to develop sustainable breeding techniques for goat reproduction in the subtropics. *Animal.* 2011, 5 (1), 74-81.

DELGADILLO, J. A., P. CHEMINEAU. Abolition of the seasonal release of luteinizing hormone and testosterone in Alpine male goats (*Capra hircus*) by short photoperiodic cycles. *Journal of Reproduction and Fertility.* 1992, 94, 45-55.

DELGADILLO, J. A., G. B. MARTIN. Alternative methods for control of reproduction in small ruminants: A focus on the needs of grazing industries. *Animal Frontiers.* 2015, 5 (1), 57-65.

DELGADILLO, J. A., 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.* 1999, 52, 727-737.

DELGADILLO, J. A., J. A. FLORES, F. G. VÉLIZ, H. F. HERNÁNDEZ, G. DUARTE, J. VIELMA, P. POINDRON, P. CHEMINEAU, B. MALPAUX. Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days. *Journal of Animal Science.* 2002, 80, 2780-2786.

DELGADILLO, J. A., M. E. CORTEZ, G. DUARTE, P. CHEMINEAU, B. MALPAUX. Evidence that the photoperiod controls the annual changes in testosterone secretion, testicular and body weight in subtropical male goats. *Reproduction Nutrition Development.* 2004, 44, 183–193.

DELGADILLO, J. A., J. A. FLORES, F. G. VÉLIZ, G. DUARTE, J. VIELMA, H. HERNÁNDEZ, I. G. FERNÁNDEZ. Importance of the signals provided by the buck for the success of the male effect in goats. *Reproduction Nutrition Development.* 2006, 46, 391–400.

DELGADILLO, J. A., S. DE LA TORRE-VILLEGAS, V. ARELLANO-SOLIS, G. DUARTE, B. MALPAUX. Refractoriness to short and long days determines the end and onset of the breeding season in subtropical goats. *Theriogenology.* 2011, 76(6), 1146-1151.

DELGADILLO, J. A., J. A. FLORES, H. HERNÁNDEZ, P. POINDRON, M. KELLER, G. FITZ-RODRÍGUEZ, G. DUARTE, J. VIELMA, I. G. FERNÁNDEZ, P. CHEMINEAU. Sexually active males prevent the display of seasonal anestrus in female goats. *Hormones and Behavior.* 2015, 69, 8-15.

DUARTE, G., J. A. FLORES, B. MALPAUX, J. A., DELGADILLO. Reproductive seasonality in female goats adapted to a subtropical environment persists independently of food availability. *Domestic Animal Endocrinology.* 2008, 35, 362-370.

DUARTE, G., M. P. HERNÁNDEZ-NAVA, B. MALPAUX, J. A. DELGADILLO. Ovulatory activity of female goats adapted to the subtropics is responsive to photoperiod. *Animal Reproduction Science.* 2010, 120, 65-70.

GONZALEZ-BULNES, A., J. A. CARRIZOSA, B. URRUTIA, A. LOPEZ-SEBASTIAN. Oestrous behaviour and development of preovulatory follicles in goats induced to ovulate using the male effect with and without progesterone priming. *Reproduction Fertility Development.* 2006, 18, 745-750.

HAFEZ, E. S. E. Studies on the breeding season and reproduction of the ewe. Part I. The breeding season in different environments. Part II. The breeding season in one locality. *The Journal of Agricultural Science.* 1952, 42, 189-231.

HAWKEN, P. A. R., G. B. MARTIN. Sociosexual stimuli and gonadotropin - releasing hormone/luteinizing hormone secretion in sheep and goats. *Domestic Animal Endocrinology.* 2012, 43, 85-94.

JORRE DE ST JORRE, T., P. A. R. HAWKEN, G. B. MARTIN. New understanding of an old phenomenon: uncontrolled factors and misconceptions that cast a shadow over studies of the 'male effect' on reproduction in small ruminants. *Turkish Journal of Veterinary and Animal Sciences.* 2014, 38, 625-636.

KARSCH, F. J., E. L. BITTMAN, D. L. FOSTER, R. L. GOODMAN, S. J. LEGAN, J. E. ROBINSON. Neuroendocrine basis of seasonal reproduction. *Recent Progress in Hormone Research.* 1984, 40, 185-232.

LASSOUED, N., G. KHALDI, Y. COGNIÉ, P. CHEMINEAU, J. THIMONIER. Effet de la progestérone sur le taux d´ovulation et la durée du cycle ovarien induits par effet mâle chez la brebis Barbarine et la chèvre locale tunisienne. *Reproduction Nutrition Development.* 1995, 35, 415-426.

LINCOLN, G. A., R. V. SHORT. Seasonal breeding: Nature´s contraceptive. *Recent Progress in Hormone Research.* 1980, 36, 1-52.

LÓPEZ-SEBASTIAN, A., A. GONZÁLEZ-BULNES, J. A. CARRIZOSA, B. URRUTIA, C. DÍAZ-DELFA, J. SANTIAGO-MORENO, A. GÓMEZ-BRUNET. New estrus synchronization and artificial insemination protocol for goats based on male exposure, progesterone and cloprostenol during the non-breeding season. *Theriogenology.* 2007, 68, 1081-1087.

MALPAUX, B., J. E. ROBINSON, M. B. BROWN, F. J. KARSCH. Reproductive refractoriness of the ewe to inductive photoperiod is not caused by inappropriate secretion of melatonin. *Biology of Reproduction.* 1987, 36, 1333-1341.

MARSHALL, F. H. A. The Croonian Lecture. Sexual periodicity and the causes which determine it. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences.* 1936, 226, 423-456.

MARTIN, G.B., C.M. OLDHAM, Y. COGNIÉ, D.T. PEARCE. The physiological responses of anovulatory ewes to the introduction of rams - a review. *Livestock Production Science.* 1986, 15, 219-247.

MARTIN, G. B., S. W. WALKDEN-BROWN, B. RACHID, S. TJONDRONEGORO, D. W. MILLER, J. S. FISHER, M. J. HÖTZEL, B. J. RESTALL, N. R. ADAMS. Non-photoperiodic inputs into seasonal breeding in male ruminants. *Perspectives in Comparative Endocrinology.* 1994, 574-585.

OLDHAM, C. M. D. T. PEARCE, S. J. GRAY. Progesterone priming and age of ewe affect the life-span of corpora lutea induced in the seasonally anovulatory Merino ewe by the 'ram effect'. *Journal of Reproduction and Fertility.* 1985, 75, 29-33.

ORTAVANT R., J. PELLETIER, J. P. RAVAULT, J. THIMONIER, N. VOLLAND, P. NAIL. Photoperiod: main proximal and distal factor of the annual cycle of reproduction in farm mammals. *Oxford Reviews of Reproductive Biology.* 1985, 7, 305-345.

PÉREZ-CLARIGET, R., M. FORSBERG, H. RODRIGUEZ-MARTINEZ. Seasonal variation in live weight, testes size, testosterone, LH secretion, melatonin and thyroxine in Merino and Corriedale rams in a subtropical climate. *Acta Veterinaria Scandinavica.* 1998, 39 (1), 35-47.

PONCE, J. L., H. VELÁZQUEZ, G. DUARTE, M. BEDOS, H. HERNÁNDEZ, M. KELLER, P. CHEMINEAU, J. A. DELGADILLO. 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. *Domestic Animal Endocrinology.* 2014, 48, 119-125.

RESTALL, B. J. The artificial insemination of Australian goats stimulated by the “buck effect”. *Proceedings of the Australian Society of Animal Production.* 1988, 17, 302-305.

RIVAS-MUÑOZ, R., 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. *Journal of Animal Science.* 2007, 85, 1257-1263.

SÁENZ-ESCÁRCEGA, P., F. G. HOYOS, G. H. SALINAS, A. J. ESPINOZA, B. A. GUERRERO, G. CONTRERAS.

SHELTON, M. Influence of the presence of a male goat on the initiation of estrous cycling and ovulation of Angora does. *Journal of Animal Science.* 1960, 19 (2), 368-375.

SIMÕES, J. Recent advances on synchronization of ovulation in goats, out of season, for a more sustainable production. *Asian Pacific Journal of Reproduction.* 2015, 4 (2), 157-165.

THIÉRY, J.C., P. CHEMINEAU, X. HERNANDEZ, M. MIGAUD, B. MALPAUX. Neuroendocrine interactions and seasonality. *Domestic Animal Endocrinology.* 2002, 23, 87-100.

VÉLIZ, F. G., C. A. MEZA-HERRERA, M. A. DE SANTIAGO-MIRAMONTES, G. ARELLANO-RODRÍGUEZ, C. LEYVA, R. RIVAS-MUÑOZ, M. MELLADO. Effect of parity and progesterone priming on induction of reproductive function in Saanen goats by buck exposure. *Livestock Science.* 2009, 125, 261-265.

WALKDEN-BROWN, S. W., B. J. RESTALL, B. W., NORTON, R. J. SCARAMUZZI, G. B. MARTIN. Effect of nutrition on seasonal patterns of LH, FSH and testosterone concentration, testicular mass, sebaceous gland volume and odour in Australian cashmere goats. *Journal of Reproduction and Fertility.* 1994, 102, 351-360.

YEATES, N. T. M. Influence of variation in length of day upon the breeding season in sheep. *Nature.* 1947, 160, 429-430.