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LA PRESENCIA DE CABRAS EN ESTRO NO EVITA EL REPOSO SEXUAL NATURAL DE MACHOS CABRÍOS, Y EL CONTACTO DIARIO DE UNA HORA O MENOS ENTRE SEXOS ESTIMULA LA ACTIVIDAD SEXUAL DE LAS HEMBRAS

Tesis

Que presenta SERGIO RAMÍREZ GÓMEZ

Como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS AGRARIAS

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Tesis

Elaborada por SERGIO RAMÍREZ GÓMEZ como requisito parcial para obtener el grado de Doctor en Ciencias Agrarias con la supervisión y aprobación del Comité de Asesoría

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RESUMEN

LA PRESENCIA DE CABRAS EN ESTRO NO EVITA EL REPOSO SEXUAL NATURAL DE MACHOS CABRÍOS, Y EL CONTACTO DIARIO DE UNA HORA O MENOS ENTRE SEXOS ESTIMULA LA ACTIVIDAD SEXUAL DE LAS HEMBRAS

Por

M.C. SERGIO RAMÍREZ GÓMEZ

Para obtener el Grado de **DOCTOR EN CIENCIAS AGRARIAS**

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Unidad Laguna

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Los objetivos de la presente fueron i) determinar si la reducción del tiempo de contacto entre machos y hembras a menos de 1 hora por día no disminuye la capacidad de respuesta ovulatoria de las hembras, y ii) determinar si el contacto de machos con hembras en estro previene la estación de reposo sexual de los machos cabríos.

Estudio 1. En este estudio se determinó la respuesta ovulatoria de cabras expuestas a 15 min diarios a machos cabríos sexualmente activos. Se utilizaron hembras y machos cabríos locales de la Comarca Lagunera, ubicada en el subtrópico mexicano (26^{o}N). En este experimento se utilizaron machos adultos que se sometieron a un tratamiento de 2.5 meses de días largos (16 h

luz/día) a partir del 1 de noviembre, seguidos del fotoperiodo natural, para estimular su actividad sexual durante el periodo de reposo (n=6). Las cabras multíparas y anovulatorias que se utlizaron se dividieron en 7 grupos: el grupo control fue aislado de los machos; los otros seis grupos se expusieron a un macho foto-estimulado (n=1/grupo) durante 15 min, 30 min, 1 h, 2 h, 4 h y 24 h por día, por 15 días consecutivos a partir del 31 de marzo (n=15 en cada grupo). Después del contacto con los machos, éstos eran retirados y alojados en corrales de descanso sin presencia de las hembras. Las cabras expuestas al efecto macho permanecieron todo el tiempo en los corrales donde se ponían en contacto con los machos. La actividad ovulatoria se determinó através de las concentraciones plásmaticas de progesterona en las muestras sanguíneas que se obtuvieron diariamente del día 1 a 15, y el día 18 después de haber introducido los machos en los grupos de hembras. Más del 90 % de las hembras expuestas a los macho ovularon, y ninguna de las hembras del grupo control ovuló ($P<0.0001$). Además, no se encontraron diferencias en el número de hembras que ovularon entre los grupos en contacto con los machos ($P>0.05$). En conclusión, 15 min de contacto diario con machos sexualmente activos es suficiente para inducir la actividad ovulatoria de las hembras en anestro. Además, estos resultados muestran claramente que en las cabras no es necesario un contacto permanente entre ambos sexos para lograr una reactivación eficiente de la actividad ovulatoria, si se utilizan machos sexualmente activos.

Estudio 2. En este estudio se determinó si la presencia permanente de cabras inducidas en estro evita la estación de reposo sexual de los machos

cabríos. Un grupo de machos se aisló de las hembras de octubre a julio; otro grupo de machos permaneció en contacto con cabras ovariectomizadas en anestro, y un tercer grupo de machos permaneció con cabras regularmente inducidas en estro. En los tres grupos de machos, las concentraciones plasmáticas de LH se determinaron una vez en octubre, febrero, marzo y junio; las concentraciones plasmáticas de andrógenos y el diámetro testicular se determinaron semanalmente desde octubre a julio. Las concentraciones plasmáticas de LH y andrógenos, así como el diámetro testicular variaron durante el estudio ($P<0.001$), pero no hubo diferencias entre los grupos de machos durante la estación de reposo sexual ($P>0.05$). En conclusión, la presencia permanente de cabras en estro no evita la aparición del reposo sexual de los machos cabríos.

Palabras claves: andrógenos, LH, hembras en anestro, hembras inducidas al estro, machos foto-estimulados.

ABSTRACT

The objectives of the present were i) to determine if the reduction of the contact time between males and females to less than 1 hour per day does not decrease the ability of female ovulatory response, and ii) determine if the contact of males with females in estrus prevents the sexual rest season of male goats.

Study 1. In this study, the ovulatory response of goats exposed to 15 min daily to male sexually active goats was determined. Female and male goats were used from the Lagunera Region, located in the Mexican subtropics (26^{o}N). In this experiment, adult males where submitted a 2.5-month long-day treatment (16 h light / day) from November 1, followed by the natural photoperiod, were used to stimulate their sexual activity during the rest period (n = 6). The multiparous and anovulatory goats that were used were divided into 7 groups: the control group was isolated from the males; the other six groups were exposed to a photo-stimulated male (n = 1 / group) for 15 min, 30 min, 1 h, 2 h, 4 h and 24 h per day, for 15 consecutive days from March 31 (n = 15 in each group). After contact with the males, they were removed and housed in resting pens without the presence of the females. Goats exposed to the male effect remained all the time in the pens where they contacted the males. Ovulatory activity was determined through the plasmatic concentrations of progesterone in the blood samples that were obtained daily from day 1 to 15, and on day 18 after the males were introduced into the female groups. More than 90% of females exposed to males ovulated, and none of the females in the control group ovulated (P <0.0001). In addition, no differences were found in the number of females that ovulated between the groups in contact with the males (P> 0.05). In conclusion, 15 min of daily contact with sexually active males is sufficient to induce the ovulatory activity of females in anestrous. In addition, these results clearly show that in goats there is no need for permanent contact between both sexes to achieve an efficient reactivation of ovulatory activity, if sexually active males are used.

Study 2. In this study it was determined if the permanent presence of estrus-induced goats avoids the sexual rest season of male goats. A group of males was isolated from females from October to July; another group of males remained in contact with ovariectomized goats in anestrus, and a third group of males remained with goats regularly induced in estrus. In the three groups of males, plasma LH concentrations were determined once in October, February, March and June; Androgen plasma concentrations and testicular diameter were determined weekly from October to July. Plasma concentrations of LH and androgens, as well as testicular diameter varied during the study (P <0.001), but there were no differences between the groups of males during the sexual rest season (P> 0.05). In conclusion, the permanent presence of goats in estrus does not prevent the appearance of sexual rest season of male goats.

Key words: androgens, LH, females in anestrus, females induced to estrus, photo-stimulated males male-female time contact.

INTRODUCCIÓN

Los animales que presentan reproducción estacional como los ovinos, caprinos y equinos, utilizan las señales ambientales para predecir la época más favorable del año para sus pariciones, y así tener éxito en el desarrollo y sobrevivencia de su progenie (Bronson y Heideman, 1994). En efecto, en cabras y ovejas de las zonas templadas y subtropicales, el fotoperiodo (horas luz/día) es el factor principal que sincroniza la actividad sexual. La manipulación artificial del fotoperiodo ha permitido desarrollar tratamientos fotoperiódicos para inducir la actividad sexual de estos pequeños rumiantes durante los periodos de reposo. Por ejemplo, en los machos cabríos expuestos a días largos artificiales (16 horas de luz/día) de noviembre a enero, seguidos del fotoperiodo natural, estimulan su actividad sexual durante la época de reposo sexual natural (marzo-abril; Delgadillo *et al.,* 2004; Ponce *et al*., 2015). Las interacciones sexuales entre machos y hembas permiten también estimular la actividad sexual de las hembras durante el anestro estacional. Esta estimulación es mayor cuando se utilizan machos fotoestimulados, sexualmente activos. Así, la introducción de los machos sexualmente activos en un grupo de hembras anéstricas inducen las ovulaciones en la mayoría de éstas, mientras que los machos sexualmente inactivos inducen ovulaciones en menos del 10% de las hembras (Delgadillo et al., 2002). Esta técnica se conoce como efecto macho. La duración del contacto entre machos y hembras puede reducirse de 24 a 1 hora por día durante 15 días, sin reducir la respuesta ovulatoria de las hembras

(Bedos *et al.,* 2014). Sin embargo, no se sabe si el contacto diario menor de una hora por día permite estimular las ovulaciones.

La introducción de una hembra en estro en un grupo de machos en reposo sexual, incrementa las concentraciones plásmaticas de LH y testosterona (Gonzalez *et al.,* 1988; Walkden-Brown *et al*., 1994a). Además, la presencia de las hembras en estro mejora el comportamiento sexual de los machos, desplegando más olfateos ano-genitales, aproximaciones, y montas con eyaculación que los machos que están en contacto con hembras en anestro (Howland *et al*., 1985; Rosa *et al.,* 2000). A este fenómeno se le llama "efecto hembra". Sin embargo, en los machos cabríos que estuvieron separados de las hembras en estro por una barrera de madera que permitía el contacto físico reducido, el contacto auditivo y visual, no evitó la estacionalidad de la secreción de testosterona ni la del diámetro testicular (Giriboni *et al.,* 2017). Es probable que el contacto fisico directo entre machos y hembras en estro pueda prevenir el reposo sexual de los machos cabríos, tal como se demostró que en las hembras caprinas, el contacto físico completo con machos sexualmente activos evitó la anovulación estacional (Delgadillo et al., 2015).

Por lo tanto, los objetivos de los estudios que conforman la presente tesis son:

1. Determinar si los machos sexualmente activos son capaces de estimular la respuesta ovulatoria de las cabras

en anestro estacional cuando el contacto diario se reduce a menos de 1 hora por día durante 15 días.

2. Determinar si el contacto continuo y total de cabras en estro con machos cabríos puede evitar la estación de reposo sexual en los machos cabríos.

REVISIÓN DE LITERATURA

1. Estacionalidad sexual

Los animales que tienen una reproducción estacional como los ovinos, caprinos y equinos, desarrollaron estrategias reproductivas para la perpetuación de sus especies. Estas estrategias permiten que los partos ocurran en la época más favorable del año para asegurar el desarrollo de las progenies (Bronson, 1985; Bronson y Heideman, 1994; Malpaux *et al.,* 1996). Por ejemplo, en los caprinos y ovinos, la duración de la gestación es de 5 meses, la actividad sexual ocurre en otoño-invierno, y los partos se producen a finales del invierno o principios de la primavera, cuando temperatura ambiental y la disponibilidad de alimento son favorables para el desarrollo de la progenie (Malpaux *et al.*, 1989; Duarte *et al.*, 2008). En cambio, en los equinos, la duración de la gestación es de 11 meses, y la actividad sexual ocurre en primaver-verano, y los partos se producen en primavera-verano (Palmer y Driancourt, 1983; Guillaume y Palmer, 1991; Nagy *et al.,* 2000).

La estacionalidad sexual se caracteriza por cambios a nivel endócrino, conductual y gonadal, y está sincronizado principalmente por el fotoperiodo, aunque las relaciones socio-sexuales pueden modificar profundamente esta estacionalidad reproductiva.

1.1. Estacionalidad sexual de las hembras caprinas

Las cabras adaptadas a latitudes templadas o subtropicales presentan estacionalidad reproductiva (Shelton, 1978; Chemineau *et al.*, 1992a; Delgadillo *et al.*, 1999; Duarte *et al.*, 2008). Esta estacionalidad se caracteriza por la alternancia entre periodos de actividad reproductiva y anestro. Así, en las cabras de raza Alpina y en las locales del norte de México, la estación sexual comienza en septiembre/octubre y termina en febrero/marzo (Figura 1; Chemineau *et al.*, 1992b; Duarte *et al.*, 2008). En las hembras caprinas, la estación sexual ocurre en otoño e invierno, mientras que el anestro en invierno y primavera. La estación sexual se caracteriza por una sucesión regular de actividad estral y ovulatoria cada 21 días, mientras que la estación de anestro se caracteriza por la ausencia total de actividad estral y ovulatoria (Chemineau *et al*. 1992b).

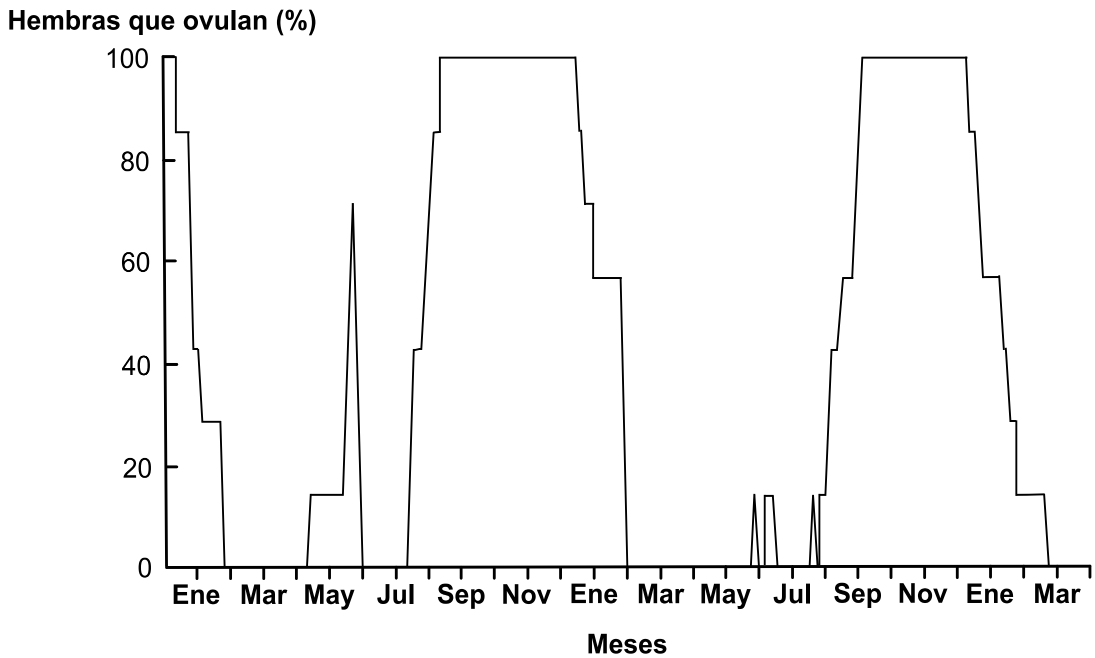

Figura 1. Variaciones estacionales de la actividad ovulatoria de cabras locales del subtrópico mexicano (26 ^{o}N) sometidas a las variaciones naturales del fotoperiodo y la temperatura ambiental (Duarte et al., 2008).

1.2.Estacionalidad sexual de los machos cabríos

Los machos cabríos adapatados a latitudes templadas y subtropicales presentan estacionalidad reproductiva. Por ejemplo, los machos de la raza Alpina o Saanen presentan un periodo de reposo sexual de marzo a agosto, mientras que la estación sexual se desarrolla de septiembre a febrero (Delgadillo *et al.,* 1991,1992). En cambio, en los machos cabríos locales del subtrópico mexicano, el periodo de actividad sexual inicia en mayo y termina en diciembre, y el periodo de reposo se presenta de enero a abril (Delgadillo *et al.,* 1999). La estacionalidad de la actividad sexual induce cambios del peso o volumen testicular, de la producción espermática y del comportamiento sexual (Santiago-Moreno *et al.,* 2013; Delgadillo *et al.,* 2004). En efecto, como consecuencia de esta estacionalidad, el peso testicular de los machos locales de la Comarca Lagunera varía de 90 g en enero-febrero que representa el periodo de reposo sexual, a 145 g en julio-agosto durante la estación sexual (Figura 2; Delgadillo *et al.,* 1999). Además, el comportamiento sexual y el olor también son modificados por la estacionalidad sexual. En los machos cabríos locales de la Comarca Lagunera y en los Cahsmere de Australia, el comportamiento sexual y la intensidad del olor son más intensos durante la estación sexual que durante el periodo de reposo sexual (Walkden-Brown *et al.,* 1994a,1997; Delgadillo *et al.,* 1999; Rivas-Muñoz et al., 2007).

Los cambios del peso testicular, del comportamiento sexual, del olor y la producción espermática son originados por las variaciones de las secreciones hormonales hipofisiarias y gonadales. En los machos de la raza Alpina y Sannen, las concentraciones plasmáticas de LH y testosterona se incrementan a partir en septiembre, para disminuir progreseivamente después hasta alcanzar sus niveles basales en febrero (Delgadillo y Chemineau, 1992). En los machos cabríos de la Comarca Lagunera, las concentraciones plasmáticas de testosterona se incrementan a partir de mayo, al inicio de la estación sexual, y disminuyen en diciembre (Figura 2; Delgadillo *et al.,* 1999).

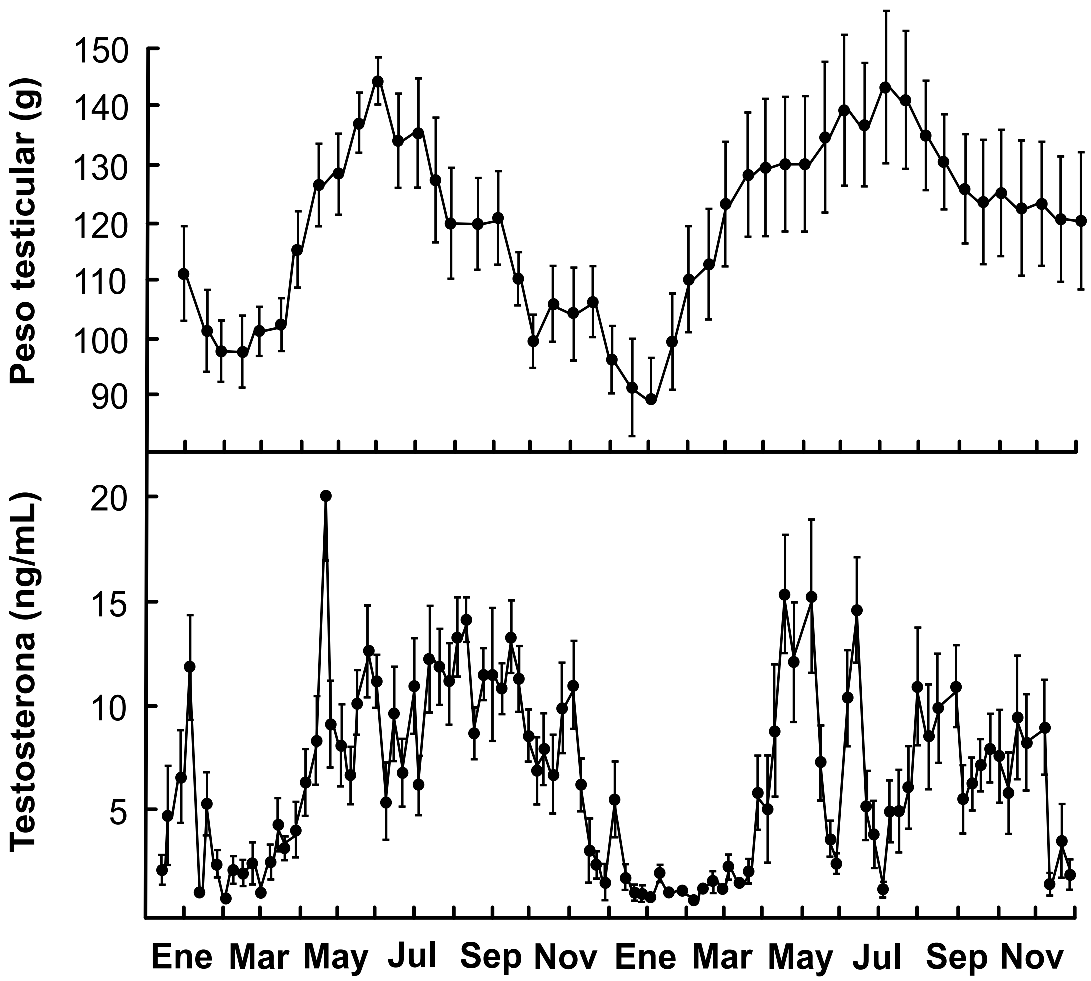

Figura 2. Variaciones estacionales (promedio ± SEM) en el peso testicular y las concentraciones plasmáticas de testosterona en machos cabríos locales del subtrópico mexicano (26 ^{o}N) sometidos a las variaciones naturales del fotoperiodo y la temperatura ambiental (Delgadillo et al., 1999).

2. Fotoperiodo

En los machos y hembras caprinos de regiones templadas y subtropicales, el fotoperiódo es el principal factor que regula la estacionalidad sexual a través de de la secreción de melatonina por la glándula pineal (Sunderland *et al.,*1995; Gatica *et al.,* 2012). En efecto, la información luminosa es captada inicialmente por la retina, en la cual se encuentran células ganglionares que expresan melanopsinas en forma de un foto-pigmento, el cual es la clave de entrada fótica que regula la función en el núcleo supraquiasmático, desde donde el impulso se transmite por vía nerviosa hasta la glándula pineal, la cual produce melatonina únicamente durante la fase obscura (Malpaux *et al*., 1997; Ebling, 2010). El perfil de secreción de la melatonina en los días cortos actúa en la pars tuberalis, que a su vez estimula las neuronas que secretan la kisspeptina. Este neuropéptido estimula la secreción del GnRH por el hipotálamo, que a su vez estimula la secreción de las gonadotropinas (LH y FSH) por la hipófisis, permitiendo que ocurra la estación sexual en machos y hembras (Dardente *et al.,* 2016). Las variaciones del fotoperiodo modulan la retroalimentación negativa de la testosterona y estradiol sobre el eje gonadotrópico, y es el mecanismo neuroendocrino responsable de los periodos de actividad y reposo sexual estacional de los caprinos (Chemineau *et al.,* 1988, Duarte *et al.* 2008). Por tanto, en los caprinos, los días cortos estimulan la actividad reproductiva y los días largos la inhiben. Así, la manipulación artificial de la duración del día permite modificar la estacionalidad reproductiva de los caprinos.

2.1. Utilización de tratamientos fotoperiódicos

Desde que se determinó que el fotoperiodo es fundamental en el control de la actividad reproductiva en los pequeños rumiantes (Bittman *et al.*, 1983), se han desarrollado tratamientos fotoperiódicos para estimular la actividad reproductiva en los periodos de reposo sexual o anestro (Chemineau *et al*., 1986; Delgadillo *et al*., 2003). Para manipular la actividad sexual de los caprinos, éstos deben de percibir alternancias de días largos artificiales y días cortos naturales, o de la inserción de 2-3 implantes de melatonina, para inducir su actividad sexual fuera de la estación sexual natural. Por ejemplo, en machos cabríos de la raza Alpina o Saanen originarios de latitudes templadas, la exposición a 2-3 meses de días largos artificiales a partir de diciembre seguidos del fotoperiodo natural o de la inserción subcutánea de 2 implantes de melatonina, estimulan la actividad sexual a partir de abril-mayo (Chemineau *et al*., 1992b; Pellicer-Rubio *et al*., 2007). De igual manera, en carneros de raza Ile-de-France, la aplicación de 2 meses de días largos artificiales a partir de enero-febrero, seguidos de la inserción de 2 implantes subcutáneos de melatonina, estimula la actividad sexual a partir de abril-mayo (Chemineau *et al*., 1992b). En los machos cabríos de la Comarca Lagunera, 2.5 meses de días largos artificiales a partir del 1 de noviembre, seguidos de fotoperiodo natural o de la aplicación de 2 implantes subcutáneos de melatonina, estimulan la secreción de LH y testosterona, así como el comportamiento sexual, la producción espermática y el olor desde finales de febrero a finales de abril, meses que corresponden al periodo de reposo sexual (Figura 3; Flores *et al.*, 2000; Delgadillo *et al*., 2001;2002; Ponce *et al*., 2014). De igual manera, Ponce *et al.* (2014) reportaron que 1.5 o 1 meses de días largos artificiales seguidos de fotoperiódo natural, estimulan la actividad sexual de los machos cabríos durante el periodo de reposo sexual, de igual manera que 2.5 meses de días largos.

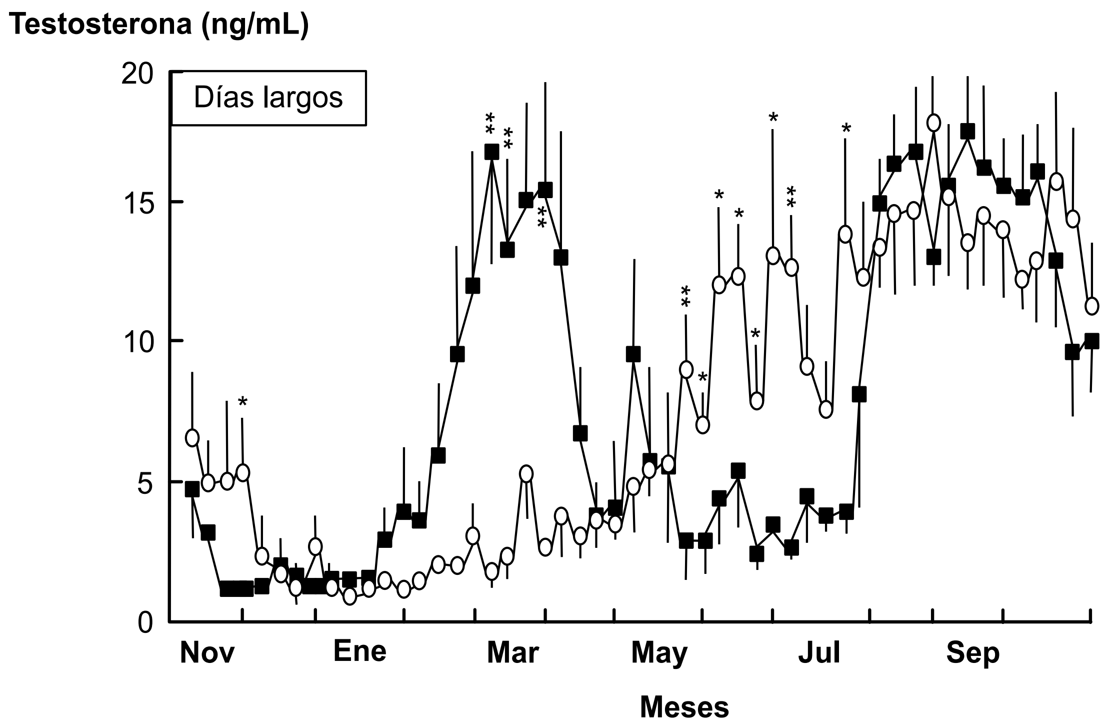

Figura 3. Concentraciones plasmáticas de testosterona (promedio ±SEM) en dos grupos de machos locales del subtrópico mexicano (26 °N) sometidos a las variaciones naturales del fotoperiodo (○) o a 2.5 meses de días largos artificiales del 1 de Noviembre al 15 de enero, seguidos del fotoperiodo natural (■).

En las hembras ovinas y caprinas, los tratamientos fotoperiódicos para estimular su actividad sexual en el anestro estacional, son similares a los usados en los machos. Así, la exposición a 2-3 meses de días largos artificiales seguidos de 3 meses de días cortos artificiales, de días crecientes o de la administración de melatonina por vía oral, subcutánea o intra-muscular, estimulan la actividad sexual en los meses que corresponden al periodo de anestro estacional (Chemineau *et al.*, 1988). Asimismo, en cabras de la raza Payoya, la exposición a 3 meses de días largos a partir del 17 de noviembre seguidos del fotoperiódo natural, estimula la actividad sexual en abril-mayo, meses que corresponden al anestro estacional (Zarazaga *et* al., 2011). Además de los tratamientos fotoperiódicos, las interacciones socio-sexuales, particularmente el efecto macho y el efecto hembra, pueden inducir la actividad sexual en los periodos de reposo.

3. Interacciones socio-sexuales entre machos y hembras

Todos los mamíferos, particularmente los que viven en grandes grupos, están inmersos en un entorno social rico y complejo que está lleno de señales auditivas, visuales y olfatorias de sus compañeros y descendientes (Wyatt, 2009). Estas señales sensoriales, en particular las señales socio-sexuales, pueden alterar profundamente los procesos fisiológicos y de comportamiento, incluida la reproducción (Martin *et al.,* 1986; Ungerfeld*,* 2007). Por ejemplo, en caprinos y ovinos la exposición de un macho sexualmente activo a una hembra en anestro induce un aumento casi inmediato de la secreción de LH, fenómeno denominado “efecto macho”.

3.1. Efecto macho

En los ovinos y caprinos, la introducción repentina de un macho en un grupo de hembras en anestro estacional puede estimular la actividad sexual de la hembra. A este fenómeno se le conoce como efecto macho (Underwood *et al*., 1944; Shelton, 1960; Delgadillo *et al.*, 2009). La respuesta de las cabras expuesta al efecto macho puede ser modificada por varios factores, como la intensidad del comportamiento sexual del macho, y la duración del contacto entre machos y hembras, entre otros (Delgadillo *et al*., 2006; 2009). Por ejemplo, la mayoría de las cabras ovulan al ser expuestas a machos sexualmente activos, mientras que un bajo porcentaje los hace al ser expuestas a machos en reposo sexual (Chasles *et al.,* 2016; Delgadillo *et al.,* 2002). De manera similar, el porcentaje de ovejas que ovulan es mayor al ser expuestas a machos que despliegan intenso comportamiento sexual, que en aquellas expuestas a machos que despliegan débil comportamiento sexual (78 % vs 95 %, respectivamente; Perkins y Fitzgerald, 1994). Por último, los carneros castrados y tratados con andrógenos que despliegan intenso comportamiento sexual son más eficaces que los machos no tratados que despliegan débil comportamiento sexual para inducir la ovulación en las ovejas (17 % vs 30 %, respectivamente; Signoret *et al.,* 1982). Estos resultados sugieren que la intensidad del comportamiento sexual desplegado por los machos es un elemento importante para estimular la actividad sexual de las cabras y ovejas.

Otro factor que modifica la respuesta de las hembras expuestas al efecto macho es el tiempo de contacto entre machos y hembras (Oldham y Pearce, 1983). Así, sólo el 18% de las ovejas ovulan cuando se exponen a los machos durante 1 día, mientras que la proporción de hembras que ovulan aumenta a 53% y al 61% cuando se exponen a machos por 24 horas por día durante 4 y 15 días, respectivamente (Signoret *et al*., 1982). En contraste, en cabras, la duración del contacto diario entre machos y hembras puede ser drásticamente disminuida sin afectar la respuesta ovulatoria, siempre y cuando se utilicen machos sexualmente activos. De hecho, todas las cabras ovularon al menos una vez cuando se expusieron 24 horas por día durante 1, 5, 10 o 15 días a los machos sexualmente activos (Ponce *et al*., 2015). Es interesante destacar que más del 89% de las cabras ovularon cuando se expusieron a los machos sexualmente activos durante 1, 2, 4 horas por día durante 15 días consecutivos, y este porcentaje no difirió de las hembras en contacto con los machos durante 24 h por día (> 95%; Bedos *et al*., 2010, 2012, 2014). Sin embargo, en las hembras expuestas a machos durante 2 o 1 hora por día, las ovulaciones se retrasaron en comparación con las que estaban en contacto con los machos durante 4 o 24 horas por día. Estos resultados demuestran que 1 h diaria de contacto con machos sexualmente activos durante 15 días es suficiente para inducir la ovulación durante el anestro estacional. Sin embargo, no se conoce si el contacto entre machos y hembras puede reducirse a menos de 1 hora por día, sin disminuir la respuesta ovulatoria.

3.2. Efecto hembra

La introducción de una hembra en estro puede modificar la estación sexual de los machos, por el efecto conocido como "efecto hembra" (Walkden-Brown *et al.,* 1994b; Zarco *et al.,* 1995; Restall *et al*., 1995). De hecho, en los carneros y machos cabríos que se encuentran en reposo sexual, las concentraciones plasmáticas de LH y testosterona se incrementan al estar en contacto directo con hembras en celo . Además, de este efecto, la presencia de las hembras en celo, mejora el comportamiento sexual de los machos los cuales muestran más olfateos, aproximaciones, flehmen y montas con eyaculación en comparación con los que están en contacto con hembras en anéstricas (Price *et al*., 1984; Gonzalez *et al.,* 1988; Walkden-Brown *et al.,* 1993b; Rosa *et al.,* 2000). Sin embargo, en los machos cabríos de la raza Gabón que estuvieron separados de las hembras en estro por una barrera de madera que permitía el contacto físico reducido, el contacto auditivo y visual no evitó la estacionalidad de la secreción de testosterona ni la del diámetro testicular (Giriboni *et al.,* 2017). Sin embargo, no se conoce si el contacto físico directo entre machos y hembras en celo pueda prevenir el reposo sexual de los machos cabríos, tal como se demostró en la hembras caprinas. En efecto, en las cabras en contacto directo con machos sexualmene activos se evitó al anovulación estacional (Delgadillo *et al.,* 2015).

Considerando los resultados bibliográficos descrito anteriormente, los objetivos de la presente tesis son los siguientes:

OBJETIVOS

1. Determinar si los machos sexualmente activos son capaces de estimular la respuesta ovulatoria de las cabras en anestro estacional cuando el contacto diario se reduce a menos de 1 hora por día durante 15 días.
2. Determinar si el contacto continuo y total de cabras en estro con machos cabríos puede evitar la estación de reposo sexual de estos.

HIPÓTESIS

1. El contacto de solo 15 min por día con machos sexualmente activos inducen la actividad ovulatoria en la mayoría de las hembras.
2. La presencia continua de hembras regularmente inducidas al estro mantenidas en contacto directo y completo con machos evita el resposo sexual estacional.

ARTÍCULOS

Artículo 1. Fifteen minutes of daily contact with sexually active male induces ovulation but delays its timing in seasonally anestrous goats.

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Artículo 2. Continuous presence of females in estrus does not prevent seasonal inhibition of LH and androgen concentrations in bucks

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1 Fifteen minutes of daily contact with sexually active male induces ovulation but delays its timing in seasonally anestrous goats

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Fifteen minutes of daily contact with sexually active male induces ovulation but delays its timing in seasonally anestrous goats

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ABSTRACT

The present experiment was conducted to determine (1) whether the sexually active bucks are able to stimulate the ovulatory activity of seasonal anestrous goats when the daily contact is reduced to 15 min/day during 15 days and (2) the exact ovulatory activity in anestrous goats exposed to bucks for 2 hours or less per day during 15 days. One group of goats (n = 15) was isolated from bucks. The other six groups (n = 15 each) were exposed to sexually active bucks (n = 1 each) for 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, or 24 hours during 15 days. Goats with plasma concentrations of progesterone greater than 0.5 ng/mL were considered to have ovulated. More than 93% of females exposed to bucks ovulated throughout the experiment regardless of the duration of contact with males, whereas none of them ovulated in the isolated group ($P < 0.0001$). The proportions of females that ovulated at least once did not differ among groups as well as the proportions of goats that displayed normal or short ovulatory cycles. The interval between the introduction of males and the first ovulation did not differ among groups of goats in contact with bucks for 15 minutes, 30 minutes, 1 hour, or 2 hours. However, in these groups, this ovulation occurred about 2 days later than in females in contact with males during 4 or 24 hours (6.3 vs. 4.4 days; $P < 0.05$). We conclude that 15 minutes of daily contact with sexually active buck is sufficient to stimulate the ovulatory activity in seasonally anestrous goats, but the first ovulation is delayed in goats exposed to males for 2 h/day or less during 15 days in comparison with those in contact with males for 4 or 24 h/day.

1. Introduction

The "male effect" is a powerful technique of sexual stimulation where the sudden introduction of a male can induce the sexual activity of female goats and ewes during the seasonal anestrus [1–3]. It is particularly useful in some breeds of goats and ewes originating from temperate or subtropical latitudes that show a reproductive seasonality

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that hinders out-of-season reproduction. In practice, the exposition of female goats and ewes to a male induces and synchronizes the secretion of LH and ovulation [4–6]. Indeed, most goats exposed to males show a preovulatory LH surge, display estrous behavior, and ovulate within the first 5 days of contact with bucks [7,8]. After this first ovulation, females show a short ovulatory cycle and ovulate again between 5 and 8 days later. This second ovulation after introduction of males is usually followed by a normal life span CL [9–11].

The ovulatory response of goats and ewes exposed to males can be influenced by the intensity of sexual behavior. Indeed, bucks rendered sexually active during the nonbreeding season by previous exposure to long days induce most females to ovulate, whereas untreated, sexually inactive ones, failed or induce few females to ovulate [12,13]. Similar results are observed in sheep. Rams displaying a high sexual behavior induce a greater proportion of ewes to ovulate, whereas rams that display a weak sexual behavior lead to a small percentage of females to ovulate [14,15].

Another factor involved in the variation of the ovulatory response of goats and ewes exposed to males is the duration of contact between sexes. In fact, only 18% of ewes ovulate when exposed to rams for 1 day, whereas the proportion of females that ovulate increases to 53% and 61% when exposed to males 24 h/day for 4 and 15 days, respectively [14]. In contrast to ewes, in goats, the duration of daily contact between males and females can be drastically decreased without affecting the ovulatory response. In fact, all goats ovulated at least once when exposed 24 h/day for 1, 5, 10, or 15 days to sexually active bucks [16]. Interestingly, more than 89% of goats ovulated when exposed to sexually active bucks for 1, 2, or 4 h/day during 15 consecutive days, and this percentage did not differ from females in contact with males for 24 h/day (>95%) [17–19]. However, in females exposed to males for 2 or 1 h/day, ovulations tended to be delayed in comparison to those in contact with males for 4 or 24 h/day. In fact, from Days 1 to 4 after introducing the males, only 14% of females exposed to males for 1 h/day ovulated, whereas 50% of those exposed to males for 4 or 24 h/day did so. Nonetheless, at Day 18 after the introduction of the males, the percentages of females that ovulated in response to the male effect did not differ significantly between females in contact with males for 1, 4, and 24 h/day [19].

In this latter study, ovulations were assessed from the pattern of daily plasma progesterone concentrations from Days 1 to 10 and on Day 18 after exposure to males. Considering the low percentage of females that ovulated in the first 4 days of contact with males and the fact that at Day 18 the proportion of females that ovulated did not differ from those remaining in contact with males 24 h/day, the pattern of secretion of plasma progesterone did not allow to determine precisely the ovulatory events in these females (i.e., when the females ovulated and the characteristics of the ovulatory cycles: short or normal ovulatory cycles).

In addition, there are no data indicating whether the sexually active bucks are able to induce ovulations in goats exposed to them for less than 1 hour for 15 consecutive days. Therefore, considering the arguments described previously, we conducted the current experiment to determine (1) whether the sexually active bucks are able to stimulate the ovulatory activity of seasonal anestrous goats when the daily contact is reduced to 15 min/day during 15 days and (2) the exact ovulatory activity in anestrous goats exposed to bucks for 2 hours or less per day during 15 days. Considering the previous results obtained with the sexually active males, we hypothesized that all durations of contact per day with sexually active bucks induce ovulatory activity of all females but that ovulation timing could be affected by the shorter durations of contact.

2. Materials and methods

2.1. Ethical note

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

2.2. General conditions of the study

The present study was conducted during the anestrous season using goats (*Capra hircus*) from the Laguna region in the state of Coahuila, Mexico (Latitude 26° 23′ N and longitude 104° 47′ O). The photoperiod in this region varies from 13 hours 41 minutes of light at the summer solstice to 10 hours 19 minutes of light at the winter solstice. In females isolated from males, the anestrous season lasts from March to August, and in males isolated from females, the sexual rest lasts from January to May [21,22]. All females were multiparous and had given birth between September and November; they were milked manually once a day during the study. Females and males were maintained in shaded open pens and 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 during the whole experiment.

2.3. Stimulation of the sexual behavior of males by exposure to long days

Six adult male goats were placed in a 10 × 5-m shaded open pen and subjected to a treatment of long days to stimulate their sexual activity during the nonbreeding season [13]. Briefly, males were exposed to artificial long days (16-hour light/8-hour darkness) from November 1, 2014 to January 15, 2015. From January 16, males were exposed to natural variations of photoperiod conditions until the end of the study (April 15). This photoperiodic treatment stimulates the secretion of testosterone from late February to late April and, as a consequence, enhances the intensity of sexual behavior and odor of males during these months corresponding to the nonbreeding season [13,16,23]. On March 23, males were individually exposed for 15 minutes to an anestrous goat, and we recorded the anogenital sniffings, nudgings, and mounting attempts [13,24,25]. All males displayed all the components of sexual behavior.

2.4. Preparation of females

All females were isolated from males from September until the onset of the study. On March 7, 17, and 27, 2015, goats were submitted to a transrectal ultrasonography to determine their ovulatory activity using an Aloka SSD-500 device connected to a transrectal 7.5-MHz linear probe [26]. Females that did not present any CL in the three ultrasonographies were considered anovulatory. None of the females presented CL, so they were all considered in seasonal anovulation.

2.5. Male effect

On March 29, 2015, the anovulatory goats were divided into seven groups (n = 15 each) balanced for body condition score (1.7 ± 0.07 each group) [27]. On March 31 (Day 0 of the study), the control group was isolated from males, whereas the other six groups were daily exposed to photostimulated males (n = 1 each) for 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, or 24 hours during 15 consecutive days. Males were introduced each day at 8 AM and removed from the females' pens after the end of each period of contact and allocated in another pen until the next day. To avoid an individual effect, males were daily rotated between groups of females. The distance between groups was more than 200 m, to avoid any interference between groups.

2.6. Measurements

The ovulatory activity was determined by plasma progesterone concentrations. To this end, daily blood samples were obtained from each female from Days 1 to 15 and on Day 18 after introduction of males. All 5-mL blood samples were collected by jugular venipuncture in tubes containing 30 μL of heparin and centrifuged at ×3000g for 30 minutes. The obtained plasma was stored at −20 °C until progesterone concentrations were determined by the immunoenzymatic assay described by Canepa et al. [28]. Sensitivity was 0.25 ng/mL. The intra-assay and interassay coefficients of variation were 7% and 10%, respectively. Females with progesterone concentrations greater than 0.5 ng/mL were considered to have ovulated [9].

2.7. Definition and statistical analysis

The characteristics of the ovulatory cycles of goats exposed to males were determined using the duration of the luteal phase [10,16]. According to the duration of high plasma concentrations of progesterone (>0.5 ng/mL), we identified two types of luteal phases: short luteal phase (2–3 days), which corresponds to a short life span CL, and long luteal phase (>4 days), which corresponds to a normal life span CL. Thus, we identified three types of ovulatory response: (1) females that displayed a short luteal phase followed by a new ovulation; these goats ovulated twice, and we consider that they displayed a short ovulatory cycle; (2) females that displayed only a short luteal phase which was not followed by any increase in progesterone concentration; these goats ovulated once and then entered in anovulation; (3) females that displayed a normal luteal phase after the first ovulation; these goats ovulated once, and we consider that they displayed a normal ovulatory cycle. The day of the first ovulation after male introduction was estimated as being the day of the first increase of progesterone above the threshold of 0.5 ng/mL for at least 2 days [16].

The total proportion of females that ovulated during the study, females that displayed short or long luteal phase, and females with short luteal phase but showing no further ovulation were analyzed using the chi-square test. When there was a statistical difference, the comparisons between groups were made using the Fisher exact test. The interval between the introduction of males and the first ovulation was compared between groups using a Kruskal–Wallis test. When there was a statistical difference, the comparisons between groups were made using the Mann–Whitney *U* test. All statistical analyses were performed using the statistical package SYSTAT 13 [29]. Results are expressed as mean ± standard error of the mean.

3. Results

3.1. Ovulatory activity

More than 93% of females exposed to the sexually active males ovulated throughout the experiment regardless of the duration of contact with males, whereas none of them ovulated in the isolated group ($P < 0.0001$; Table 1). Interestingly, the proportions of females that ovulated at least once did not differ between groups of females that were in contact with males for 15 min, 30 min, 1 h, 2 h, 4 h, or 24 h/day (Table 1). In addition, the proportions of goats that displayed a normal or short ovulatory cycle did not differ among groups. Similarly, the proportions of goats that entered in anovulation after their luteal phase of short duration did not differ among groups (Table 1).

In goats isolated from males, plasma concentrations of progesterone remained low throughout the study, indicating anovulation. By contrast, in goats exposed to males, plasma concentrations of progesterone increased at least once over 0.5 ng/mL of plasma during the sampling series, indicating ovulation (Fig. 1). Indeed, in goats that displayed a short ovulatory cycle, concentrations of progesterone increased in two occasions, the first time during Days 4 to 8 and the second one during Days 10 to 14 after the introduction of males (see goat 428). On the contrary, in goats that displayed a normal ovulatory cycle after the first ovulation induced by bucks, concentrations of progesterone increased during one occasion during Days 4 to 12 after introduction of males and remained high until the end of study (see goat 1). Finally, in goats that ovulated once and stopped ovulating thereafter, progesterone increased only during 1 or 2 days, then remained low throughout the study (see goat 64).

A noticeable difference between groups appeared when considering the interval between the introduction of males and the first ovulation ($P < 0.0001$). Indeed, the interval elapsed between the introduction of males and the first ovulation was about 2 days greater in the groups in contact with males for 15 minutes, 30 minutes, 1 hour, or 2 hours,

Table 1
Characteristics of the ovulatory response of seasonally anestrous goats isolated from bucks or exposed to sexually active males for 15 min, 30 min, 1 h, 2 h, 4 h, or 24 h during 15 consecutive days.

Groups	n	Females with ovulations (%)	Interval to the first ovulation (days)	Goats with normal luteal phase (%)	Goats with short luteal phase followed by a new ovulation (%)	Goats with short luteal phase followed by anovulation (%)
Isolated	15	0/15 (0)^{a}	—	—	—	—
15 min	15	14/15 (93)^{b}	5.7 ± 0.5^{a}	0/14 (0)	13/14 (93)	1/14 (7)
30 min	15	14/15 (93)^{b}	6.3 ± 0.5^{a}	2/14 (14)	12/14 (86)	0/14 (0)
1 h	15	14/15 (93)^{b}	7.1 ± 0.7^{a}	2/14 (14)	11/14 (79)	1/14 (7)
2 h	15	14/15 (93)^{b}	6.2 ± 0.4^{a}	0/14 (0)	14/14 (100)	0/14 (0)
4 h	15	15/15 (100)^{b}	4.6 ± 0.2^{b}	3/15 (20)	12/15 (80)	0/15 (0)
24 h	15	15/15 (100)^{b}	4.2 ± 0.2^{b}	4/15 (27)	10/15 (66)	1/15 (7)

Males were rendered sexually active by exposure to long days (16 hours of light per day) from November 1 to January 15 followed by natural photoperiodic conditions.
^{a,b}Different superscripts within each column indicate significant difference ($P < 0.05$).

compared with those in contact with males during 4 or 24 hours ($P < 0.01$). However, this interval did not differ between groups of females in contact with males for 15 minutes, 30 minutes, 1 hour, or 2 hours, or between groups in contact with males for 4 or 24 hours (Table 1).

4. Discussion

Our results show that the photostimulated, and therefore sexually active bucks, were able to stimulate the ovulatory activity of goats during the seasonal anestrous, even when the daily contact between males and females was reduced to 15 min/day during 15 consecutive days. In fact, the proportions of goats that ovulated did not differ among groups exposed to males for 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, or 24 hours. In addition, our results show that the interval between the introduction of males and the first ovulation was delayed in females in contact with males for 2 hours or less compared with those in contact with males for 4 or 24 hours. Unambiguously, our findings and those published previously by our team [17,19] clearly show that in goats, a permanent contact between both sexes is not necessary to achieve an efficient reactivation of the ovulatory activity, if sexually active males are used.

In the present study, the proportions of goats that ovulated and the proportions of goats that displayed short or normal ovulatory cycles did not differ among groups, regardless the duration of contact with males. Our data are in agreement with the results reported by Bedos et al. [17,19], where goats remained in contact with males for 1, 2, 4, or 24 h/day during 15 days. Therefore, the main outcome of our study is that 15 daily minutes of contact with sexually active males was enough to induce ovulations in most goats. The latter results strongly suggest that this shortened daily duration of contact with males was enough to reactivate the GnRH-LH secretion and therefore allowing ovulation to occur [8,30].

It is likely that the success of short-duration contact between males and females to reactivate the ovulatory activity is related to the use of sexually active males, and several studies agree with this hypothesis. First, the sexually active bucks induced more females to ovulate than the untreated bucks, which displayed a weak sexual behavior [26]. Second, the sexually active bucks stimulated and maintained high LH secretion allowing ovulation to occur in most females. On the contrary, the sexually inactive bucks only induced a short-term elevation of LH secretion avoiding ovulation to occur in the females exposed to them [8,31]. In the present study, the bucks were rendered sexually active by exposure to long days to increase their plasma testosterone concentrations, odor, and sexual behavior during the nonbreeding season, when the present study was performed. Therefore, we can conclude that the use of the sexually active males was an important element to reactivate the ovulatory activity of goats even when the daily contact was reduced to 15 daily minutes.

A delay in the first ovulation after introduction of males was observed when the duration of contact between sexes was reduced. Indeed, the interval between the males' introduction and the first ovulation was longer in females in contact with males for 15 minutes, 30 minutes, 1 hour, or 2 hours, in comparison to the females being in contact with males for 4 or 24 hours. Our findings agree with those reported in goats exposed to males for 2 or 1 hour, in which ovulation was delayed in comparison with those in contact with males for 4 or 24 hours [19]. In our study, the delayed ovulation in goats in contact with males for 2 hours or less strongly suggests that the short duration of contact between both sexes probably provided a weaker stimulation. In the present study, we did not determine LH secretion, but we can speculate that the pattern of secretion of this hormone was similar of that described by Bedos et al. [19]. Indeed, in goats exposed to bucks for 2 h/day during 15 consecutive days, LH secretion increased each day at male introduction and decreased when males were removed; this pattern of LH secretion induced most of female to ovulate. By contrast, in goats exposed to males 24 h/day, LH secretion increased at male introduction and remained elevated until ovulation [19]. Therefore, our findings indicate that a short duration of contact ranging from 15 minutes to 2 hours is sufficient to induce ovulations in seasonal anovulatory goats, but these durations of contact between both sexes probably provided a weaker stimulation of the ovaries by LH secretion, delaying the first ovulation after male introduction.

In the study of Bedos et al. [19], they did not describe the characteristics of the ovulatory response of females

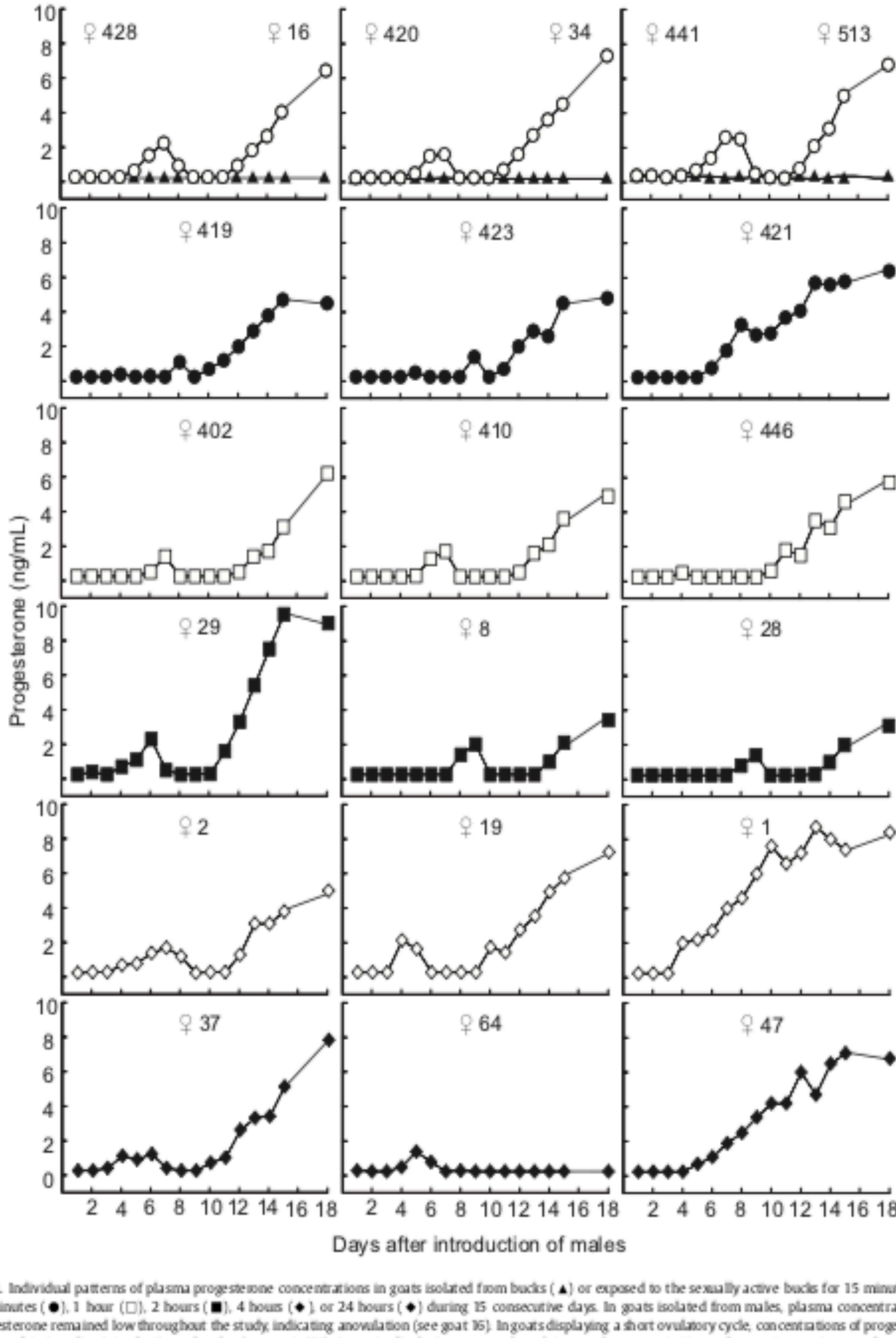

Fig. 1. Individual patterns of plasma progesterone concentrations in goats isolated from bucks (▲) or exposed to the sexually active bucks for 15 minutes (○), 30 minutes (●), 1 hour (□), 2 hours (■), 4 hours (◆), or 24 hours (◆) during 15 consecutive days. In goats isolated from males, plasma concentrations of progesterone remained low throughout the study, indicating anovulation (see goat 16). In goats displaying a short ovulatory cycle, concentrations of progesterone increased twice after introduction of males (see goat 428). In goats displaying a normal ovulatory cycle, concentrations of progesterone increased once after introduction of males and then remained high until the end of study (see goat 1). Finally, in goats that ovulated once and entered in anovulation, progesterone increased only 1 to 2 days, then decreased and remained low throughout the study (see goat 64). Bucks were rendered sexually active by exposure to artificial long days (16 hours of light per day) from November 1 to January 15 followed by natural photoperiodic conditions.

exposed to males for 1 or 2 hours during 15 days. In the present study, we were able to describe completely the ovulatory response of goats exposed to males when duration of contact was dramatically reduced. Indeed, a great proportion of goats in which the first ovulation was delayed displayed short ovulatory cycles, and this proportion did not differ from those in contact with males for 4 or 24 hours. In our study, the proportions of goats displaying short ovulatory cycles agree with previous studies that used the photostimulated bucks [26].

The determination of the ovulatory response of goats has important practical applications. In fact, most females that were exposed to males for 2 hours or less displayed a short ovulatory cycle, but the second luteal phase was of normal duration. Therefore, our findings strongly suggest that these goats could become pregnant even when the duration of contact with males is as short as 15 min/day, if males would remain with females for more than 15 days.

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2 Continuous presence of females in estrus does not prevent seasonal inhibition of LH and androgen concentrations in bucks

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Continuous presence of females in estrus does not prevent seasonal inhibition of LH and androgen concentrations in bucks

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In male goats, being in permanent visual contact with females in estrus does not prevent seasonal variation in certain endocrine hormone levels and sexual activities. In this study, we tested whether continuous and full contact with females in estrus prevented seasonal endocrinological variation in bucks. In 1 experiment (Exp. 1), we verified that the sudden introduction of goats in estrus increased the plasma concentrations of androgen in bucks during the nonbreeding season under our experimental conditions. In another experiment (Exp. 2), we tested the ability of estrous goats to prevent seasonal inhibition of LH and androgen secretions in bucks kept in permanent and full contact with them. In Exp. 1, 3 groups of bucks (n = 5 in each group) were isolated from females from the months of July to January. On January 27, one group continued being isolated from females; a second group was exposed to ovariectomized, untreated goats; and a third group was exposed to ovariectomized goats with induced estrus. Plasma androgen concentrations were determined every 2 h from 8 h before to 8 h after the introduction of females. The introduction of estrus-induced goats significantly increased androgen concentrations, which were higher than in the isolated bucks, as well as in those exposed to untreated goats ($P < 0.05$). In Exp. 2 (n = 5 per group), one group of bucks was isolated from females from October to July, whereas two other groups remained in contact with ovariectomized goats, either untreated or regularly induced to estrus. In the three groups of bucks, plasma concentrations of LH were determined once during the months of October, February, March, and June, whereas androgen concentrations were determined weekly from October to July. The mean plasma LH and androgen concentrations were low and did not differ among the groups of bucks during the normal seasonal period of sexual inactivity ($P > 0.05$). We conclude that full contact and sexual interactions with estrus-induced goats failed to stop the seasonality of LH and androgen plasma concentrations of bucks, although bucks could respond to the introduction of females by acute increases in plasma LH and androgen.

1. Introduction

Bucks and rams from the subtropical latitudes are seasonal breeders that exhibit sexual activity in summer and autumn and sexual quiescence in winter and spring

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[1,2]. This seasonality is primarily controlled by annual changes in photoperiod [3,4]. However, in goats and sheep, sociosexual interactions between males and females can modify the timing of both the onset and end of the breeding season by phenomena known as the "male effect" and "female effect" [5]. In female sheep and goats, the introduction of a male to a group of females, known as the "male effect," can stimulate the secretion of LH and lead to ovulation [6,7]. Two factors are important for the male effect to occur. First, the odor of the male is important for triggering the initial stimulation of LH secretion that will lead to ovulation [8]. The second factor has to do with the behavioral activity of the male. Our goat breeds only responded to bucks that were sexually active, and the initial surge of LH failed to be maintained if does were kept with sexually active bucks when the bucks were anesthetized [9,10]. In fact, sexually active bucks are such a powerful stimulus that their continuous presence in a group of females overrides the seasonal inhibition of reproductive activity, and does will keep cycling during the normal period of anestrus [11].

Sociosexual interactions also influence the reproductive physiology and behavior of bucks and rams. Bucks and rams exposed to females in estrus during the breeding and nonbreeding seasons show an increase in plasma LH and testosterone concentrations, a phenomenon known as the "female effect" [12–14]. In males of both species, testosterone levels remain high from 3 to 12 h after their initial contact with females in estrus and decrease thereafter [12,13,15]. In addition, in these species, the plasma levels of testosterone are higher in males having visual contact with females in estrus than in those having visual contact with females in anestrus [16,17]. However, continuous visual contact over a 13-mo period with estrus-induced females failed to modify the seasonal pattern of sexual activity in bucks, although it improved testicular activity, that is, enhanced semen quality and increased testicular fluid content [18].

Considering reports in the literature concerning (1) the effect of females in estrus on LH, testosterone, and testicular function in males, (2) the importance of full behavioral interactions between the sexes for a long-lasting response, and (3) the inhibition of seasonal anestrus in does by the continuous presence of sexually active bucks, we hypothesized that the continuous presence of estrous does in direct and full contact with bucks would prevent the normal decline in sexual activity of bucks. This would be consistent with our findings in does [11] and in contrast to what has been reported for bucks that have only maintained visual contact with does [18]. To verify this, we carried out 2 experiments. In Exp. 1, we confirmed that under experimental conditions, bucks responded to the presence of females in estrus by showing an increase in androgen secretion. This was a necessary step before interpreting the results of the second experiment. In Exp. 2, we investigated whether bucks in permanent and direct physical contact with females in estrus experienced an inhibition of the seasonal variations in LH, androgen, and scrotal circumference (SC) associated with sexual rest.

2. Materials and methods

2.1. Ethical note

The experimental procedures used in the present study were in accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals [19].

2.2. General conditions of the study

The present study was conducted using local goats (*Capra hircus*) from the Laguna region in the state of Coahuila (Latitude 26° 23′ N and Longitude 104° 47′ W) in subtropical northern Mexico. These goats resulted from crosses between the Spanish Granadina, Murciana, and Malagueña breeds, which were further crossed with Alpine, Saanen, Toggenburg, and Anglo-Nubian breeds within the last 50 yr. In females isolated from males, the anestrous season lasts from March to August, and in males isolated from females, the decrease in sexual activity lasts from January to May [1,20]. All animals were kept in shaded, open pens, and fed daily with 2 kg of alfalfa hay (17% CP) and 200 g of commercial concentrate (14% CP; 1.7 Mcal/kg) during the whole study. They had free access to water and mineral salts.

2.3. Experiment 1

2.3.1. Females

We used multiparous, ovariectomized goats that were induced into estrus ($n = 3$) or not ($n = 3$) by the subcutaneous introduction of 1 mg of estradiol-17ß preceded by injections of 5 and 10 mg of progesterone, respectively, 96 and 72 h before the injection of estradiol (Syva laboratory, León, Spain). This hormonal treatment stimulates estrus behavior and receptivity in ovariectomized goats [21].

2.3.2. Males

We used sexually experienced male goats that were aged 3 yr at the beginning of the study. These males remained isolated from females from July to January and did not have visual, auditory, or olfactory communication with females, as the does were on another farm 20 km away. In January, the males were divided into 3 groups ($n = 5$ each) that were evenly matched according to BCS and SC. These groups were allocated to 3 different shaded, open pens (5×7 m). The control group (BCS: 2.4 ± 0.2; SC: 29 ± 0.9 cm) continued to be isolated from females. However, on January 27, the second group (BCS: 2.5 ± 0; SC: 28 ± 0.7 cm) was put in contact with ovariectomized goats, whereas the third group (BCS: 2.5 ± 0; SC: 28 ± 0.8 cm) was put in contact with ovariectomized goats that had been induced to estrus. The 3 groups of males could not see each other, as the distance between groups was more than 150 m; this prevented any risk of interference between groups [12]. In hormonally treated goats, estrous behavior was verified before their introduction to the corresponding group of bucks. This was accomplished by placing each female in contact for 5 min with a male that had been fitted with a harness. Females that continued standing while being mounted by the male was considered as evidence of estrous behavior [22]. As expected, a pilot experiment verified that in the presence of estrous goats, contrary to what occurs with anestrous goats, bucks demonstrated more pronounced sexual behavior (data not shown).

2.3.3. Measurements

Plasma concentrations of testosterone were determined before (−8, −4, −2 h) and after (2, 4, 8 h) the introduction of females to each group of males (0 h). All blood samples were collected by jugular venipuncture in 5 mL tubes containing 30 μL of heparin. Plasma was obtained after centrifugation at 2,500 × g for 20 min and stored at −20°C until hormonal determinations could be performed. The concentration of testosterone was determined using a direct RIA method derived from Garnier et al [23] and Hochereau-de-Reviers et al [24]. For each sample, 50 μL of plasma was assayed in duplicate. Buffer containing 7.5 nCi-labeled testosterone and 0.5 μg of rabbit γ-globulins and buffer with a specific antiserum from rabbits (diluted at 1/45,000) were added to each tube. The antiserum cross-reacted with dihydrotestosterone (43%), androstenediol (4.5%), androstenedione (4.5%), androstanediol (3.5%), and less with other steroids (0.1%). The minimum level of quantification was 0.06 ng/mL. All samples were assayed using the same assay. The intra-assay CVs were 20.3%, 7.1%, and 12.1% for samples containing 0.6, 2.3, and 4.2 ng/mL of testosterone, respectively. Given the level of cross-reaction with other androgens in the assay, we used the more general term "androgens" when referring to the results of the present experiments.

2.4. Experiment 2

2.4.1. Females

We used 2 groups of multiparous, ovariectomized female goats, which were kept in 2 different shaded, open pens (5 × 5 m) adjacent to the males. These pens only allowed auditory and olfactory contact between the sexes. One group of females (n = 9) remained untreated, whereas the other group (n = 9) was induced to estrus (3 females at a time) every 3 wk, as described in Exp. 1. One month before the onset of the study, we determined from 3 of the 9 goats that the duration of estrus in unmated females was 147.4 ± 8.5 h. Afterward, in November and February, this duration was reduced to 94.4 ± 10.2 h in goats that were mated and kept in contact with males. These 9 hormonally treated females, used in groups of 3 estrus females at a time, were introduced to males every week, such that males were in the presence of estrous goats for 4 (94.4 h) to 6 (147.4 h) d each week. The same females were used repeatedly, ensuring that any effect of their presence would be because of their sexual activity rather than to some effect of novelty.

2.4.2. Males

We used 3 groups of sexually experienced male goats that were aged 3 to 4 yr at the beginning of the study (n = 5 per group). From October to July, the control group was isolated from females. The second and third groups remained in continuous and full contact with ovariectomized females that were either untreated or induced to estrus, respectively. The untreated and induced-estrus goats were exchanged, in groups of 3, within their respective groups of bucks every week. Thus, bucks of each group were always in contact with 3 females at any given time. The distances among the 3 groups of males were more than 200 m, and visual contact between them was not possible, preventing any risk of interference between groups.

2.4.3. Measurements

Plasma LH was determined every 20 min for 6 h (08:00 AM–2:00 PM) in October, February, March, and June. All the blood samples were taken 48 h after the weekly exchange of 3 females in estrus for new ones. Thus, there were always females in estrus at the time of blood sampling, as estrus lasted for 5 to 6 d (see Section 2.4.1). Each sample was analyzed in duplicate using a double-antibody RIA previously described by Pelletier et al [25] and Caraty et al [26]. Assay sensitivity was 0.1 ng/mL. The intra-assay CV was 5.2%, and the inter-assay CV was 11.0%. All samples were assayed using the same assay. LH pulses were detected using the DynPeak algorithm [27]. Plasma concentrations of androgen were determined once a week from October to July, as described in Exp 1. All blood samples were taken 48 h after the weekly exchange of 3 females in estrus for new ones. SC was determined for each male once a week using a tape measure graduated in centimeters and with millimetric precision. Light pressure was used on the tape to pull the testes together, and the equatorial region of both testicles was measured [28]. This allowed for the estimation of testicular weight, which is a good indicator of spermatogenic activity [1]. Sexual interactions were not quantified due to the very high number of sociosexual interactions between animals in the third group when males and females were put together, and weekly when females were replaced in the last 2 groups.

2.5. Statistical analysis

In Exp. 1, plasma androgen concentrations were analyzed using 2-way repeated-measures ANOVA to detect differences between treatments. The model included treatment (group), sampling time (hours) and the interaction between these factors. The Tukey test was used for post hoc comparisons. In Exp. 2, LH pulse frequencies were compared over time using the Friedman test with repeated measures (months), followed by the Kruskal–Wallis test to compare groups within months [29]. To analyze plasma LH and androgen concentrations and SC, we calculated monthly means for each animal. Thereafter, these means were analyzed using 2-way repeated-measures ANOVA to detect differences between treatments. The model included treatment (groups), the sampling day (months), and the interaction between these factors. The Tukey test was used to make post hoc comparisons when there were significant interactions. Analyses were computed using the statistical package SYSTAT 13 [30]. Data were expressed as the mean ± SEM, and differences were considered significant at the level of $P \leq 0.05$.

3. Results

3.1. Experiment 1

3.1.1. Patterns of androgen secretion

The plasma androgen concentrations of bucks varied over time ($P < 0.001$), and there was an interaction between time and groups ($P < 0.001$), indicating that profiles of plasma androgen concentrations varied differently over time between treatments. Plasma androgen concentrations decreased progressively from −8 to 0 h, and these concentrations did not differ among groups of bucks before the introduction of females. Thereafter, in bucks exposed to goats in estrus, androgen concentrations significantly increased 2 h after the introduction of females and remained high during the 8-h sampling period. Androgen concentrations in this group were significantly higher than in control bucks and in bucks in contact with untreated ovariectomized females ($P < 0.01$), and there were no differences between the latter 2 groups of bucks (Fig. 1).

3.2. Experiment 2

3.2.1. Patterns of LH secretion

Luteinizing hormone pulse frequencies varied over time in the 3 groups of bucks ($P < 0.05$), but there were no significant differences between the groups when compared within months ($P > 0.05$). The overall mean for the LH pulse frequency was similar in bucks from the control group (1.3 ± 0.1) and those in contact with untreated (1.9 ± 0.4) or estrus (1.5 ± 0.2) females. Mean LH plasma concentrations varied over time ($P < 0.001$), but there was no significant interaction between time and groups of males ($P > 0.05$), indicating that the profiles of plasma LH concentrations did not differ over time between treatments. The mean LH plasma concentrations were, in all cases, lower than 1 ng/mL in the 3 groups of bucks (Fig. 2).

3.2.2. Patterns of androgen secretion

Mean plasma androgen concentrations varied over time ($P < 0.001$), and there was a significant interaction between time and the groups of bucks ($P < 0.05$), indicating that the patterns of plasma androgen concentrations differed over time between treatments. In December, the mean plasma androgen concentrations tended to be higher in bucks in contact with ovariectomized, untreated females than for

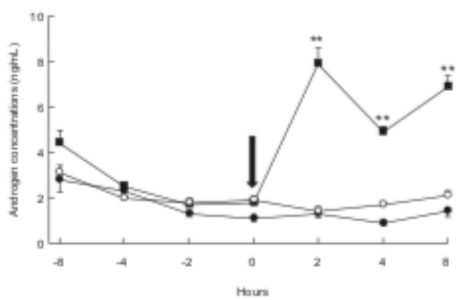

Fig. 1. Plasma androgen concentrations (mean ± SEM) in 3 groups of mixed breed male goats (n = 5 per group). One group remained isolated from goats (●), the second one was exposed to ovariectomized, untreated goats (○), and the third group was exposed to ovariectomized, estrus-induced goats (■). ↓ indicates the moment of introduction of untreated or estrus-induced females to the 2 groups of bucks. ** indicates a significant difference among groups at a given sampling point ($P < 0.01$).

those in contact with females in estrus ($P = 0.052$). However, in July, mean plasma androgen concentrations were higher in control bucks than those in contact with ovariectomized and untreated or estrus females ($P < 0.01$), but there were no differences between the last 2 groups ($P > 0.05$; Fig. 3A). Finally, mean plasma androgen concentrations were lower and did not differ between groups from January to June ($P > 0.05$), during the nonbreeding season.

3.2.3. Scrotal circumference

The mean SC varied over time ($P < 0.001$), but there was no significant interaction between time and groups of males ($P > 0.05$). The SC decreased in the 3 groups of males from October, reaching its lowest values in January. Thereafter, it increased progressively in all groups, reaching their maximum values in July (Fig. 3B).

4. Discussion

The results from Exp. 1 demonstrate that under our experimental conditions, sexually inactive bucks responded to the sudden exposure of females induced to estrus by increasing their plasma concentrations of androgen, as has already been reported for other breeds and conditions [12–14]. By contrast, the results from Exp. 2 failed to support our hypothesis that the permanent presence of goats induced to estrus would prevent seasonal variation in LH and androgen concentrations or changes in SC. Indeed, the response of bucks exposed to full and continuous interaction with females in estrus did not differ from that of bucks kept with ovariectomized, untreated females or from those kept completely isolated from females. It is unlikely that this lack of difference is due to some incapacity of our males to respond under our conditions because a response to females in estrus was clearly present in Exp. 1. Rather, it indicates that contrary to our hypothesis, continuous full contact and behavioral interactions with sexually receptive females does not override the inhibitory effect of photoperiod on seasonal changes associated with buck reproduction. This is in contrast to our previous results for female goats, in which the continuous presence of active bucks overrode the inhibitory action of photoperiod on reproductive seasonality [11]. Nevertheless, the results of Exp. 2 are congruent with those of Giriboni et al [18] and indicate that the lack of effect of females in estrus on the general pattern of reproductive activity of bucks was probably not a result of the lack of full contact between the sexes. Although we did not quantify the interactions between and among the sexes in the 3 groups (see Section 2.4.3.), our direct visual observations during Exp. 2 suggest that intense sexual relationships occurred between males and females, especially in the group of males that were in the continuous presence of estrous goats that were changed on a weekly basis. Thus, it is unlikely that the absence of an effect of the presence of estrous goats on the seasonal activity of bucks was due to a lack of sexual interactions between bucks and does. As a whole, our results are congruent with previous reports investigating bucks isolated from females in the subtropical latitudes [1]. Taken together, these findings suggest that full or partial physical contact with estrus-induced goats does not prevent the

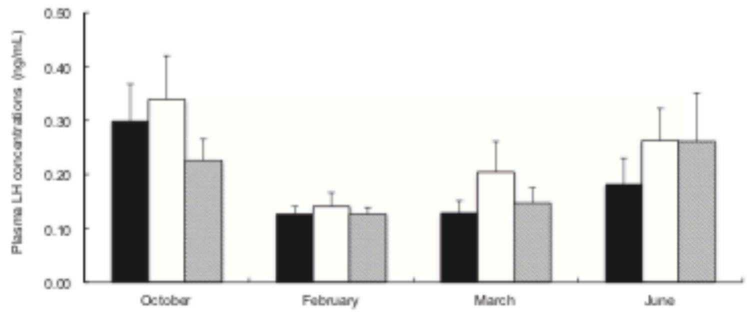

Fig. 2. Plasma LH concentrations (mean ± SEM) in 3 groups of mixed breed male goats (n = 5 per group). One group was isolated from goats (▨), the second one was in continuous contact with ovariectomized, untreated goats during the study (□), and the third group was in continuous contact with ovariectomized, estrus-induced goats during the study (■). The untreated and estrus-induced goats were exchanged each week within their respective groups of bucks, and bucks in each group were always in contact with 3 females.

seasonal inhibition of the hypothalamus-pituitary-gonadal axis activity in bucks.

The results of Exp. 2 do not provide evidence that the permanent presence of goats induced to estrus stimulated a long-lasting secretion of LH and androgens. It is unlikely that this lack of response was due to a general unresponsiveness of bucks, as a short-term response of LH and androgens was clearly present in Exp. 1. One possible explanation for the lack of a sustained response in the bucks in Exp. 2 could be a lack of novelty concerning the stimulus females, with their repeated use leading to habituation and extinction of the hormonal response. Novelty is known to facilitate sexual activity in male goats [31]. However, whether this was the case in the present

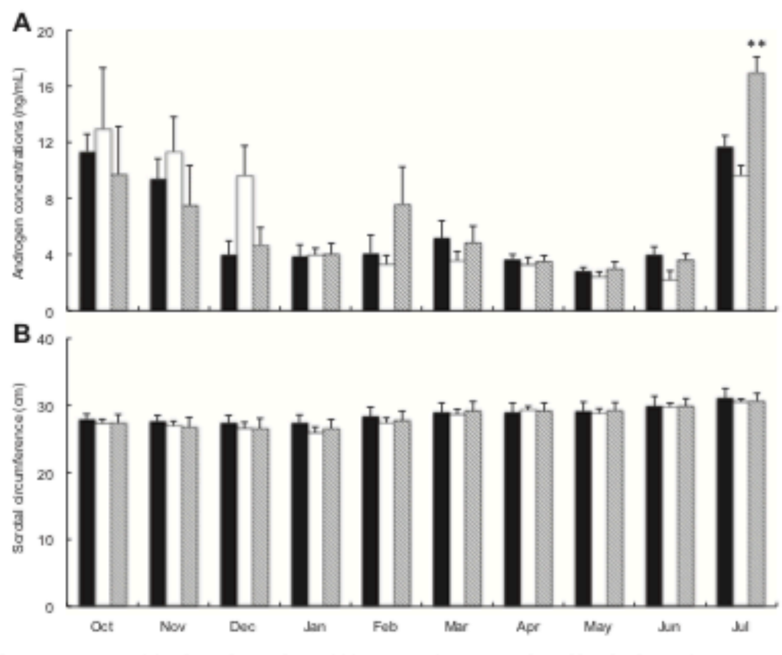

Fig. 3. Plasma androgen concentrations (A) and scrotal circumference (B) (mean ± SEM) in 3 groups of mixed breed male goats (n = 5 per group). One group was isolated from goats (▨), the second one was in continuous contact with ovariectomized, untreated goats during the study (□), and the third group was in continuous contact with ovariectomized, estrus-induced goats during the study (■). The untreated and estrus-induced goats were exchanged each week within their respective groups of bucks, and bucks in these groups were always in contact with 3 females. ** indicates a significant difference between groups ($P < 0.01$).

experiment warrants further investigation, especially as novelty was clearly not a limitation used to override seasonal anestrus in goats stimulated by sexually active bucks [11]. In addition, the lack of increases in LH and androgen concentrations in our bucks 48 h after exchanging females in Exp. 2 is congruent with results reported in rams, in which the concentrations of LH and testosterone decreased after their initial rise, even in males that remained in contact with females in estrus, independent of any habituation [13,15].

The only marked difference between groups was a higher concentration of androgens at the end of July in the group of bucks isolated from females, which may appear contrary to what was initially expected. In fact, this difference occurs when bucks normally start their seasonal sexually active period, and it certainly marked the seasonal onset of normal sexual activity in our bucks [1]. In the group of bucks isolated from females, we noted that the first male to become sexually active directed his sexual activity toward the other males of the group, which resulted in an entraining effect on the other males. This was obvious to the experimenters, although this behavior was not quantified. In contrast, in the 2 groups with females present, the first male to be sexually active directed his activity toward the females in the group, rather than directing it toward the other males. Therefore, the overall sexual activity of all the males in these 2 latter groups was paradoxically slower to start than in the group of isolated males; hence, the higher concentrations of androgens found in isolated bucks at the end of July. To our knowledge, such an entraining effect has not been reported in males but has been documented in females [32]. Further experimentation is currently underway to investigate this possibility.

In conclusion, there appears to be a clear difference between does and bucks as reproductive biostimulators. Indeed, the possibility of bucks sexually interacting continuously with females in estrus did not prevent the normal season-dependent decrease in sexual activity in males during this experiment. In contrast, the permanent presence of sexually active bucks for whom sexual activity had been maintained by a photoperiod treatment, prevented the seasonal decrease of LH plasma concentrations in ovariectomized goats bearing a subcutaneous estradiol implant [33] under similar latitudes and with the same breed of animal. Therefore, in goats, the continuous sexual biostimulation of one sex by sexually active partners of the other sex appears able to prevent sexual seasonality in females, but not in males. In sum, the annual photoperiodic inhibition of LH secretion is more easily overridden by sociosexual stimuli in does than in bucks. Bucks appear to depend more strictly on the duration of day-length than females, thus being the primary biostimulus controller of seasonality in this species.

CRediT authorship contribution statement

S. Ramírez: Conceptualization, Methodology, Data curation, Visualization, Formal analysis, Writing - review & editing. **D. Chesneau:** Conceptualization, Investigation, Methodology, Writing - review & editing. **E. Grimaldo-Viesca:** Conceptualization, Methodology, Data curation, Visualization, Formal analysis, Writing - review & editing. **J. Vielma:** Conceptualization, Methodology, Data curation, Visualization, Formal analysis, Writing - review & editing. **H. Hernández:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing. **J. Santiago-Moreno:** Conceptualization, Investigation, Methodology, Writing - review & editing. **P. Chemineau:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing. **M. Keller:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing. **J.A. Delgadillo:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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DISCUSIÓN GENERAL

En el estudio 1, los resultados muestran que los machos foto-estimulados, sexualmente activos, fueron capaces de estimular la actividad ovulatoria de las cabras durante el anestro estacional, incluso cuando el contacto diario entre machos y hembras se redujo a 15 min por día durante 15 días consecutivos. De hecho, las proporciones de cabras que ovularon no difirieron entre los grupos expuestos a los machos durante 15 minutos, 30 minutos, 1 hora, 2 horas, 4 horas o 24 horas. Además, nuestros resultados muestran que el intervalo entre la introducción de los machos y la primera ovulación se retrasó en las hembras en contacto con los machos durante 2 horas o menos en comparación con las que estuvieron en contacto con los machos durante 4 o 24 horas. Sin ambigüedad, nuestros hallazgos y los publicados previamente por nuestro equipo (Bedos *et al.,* 2010; Bedos *et al.,* 2014) muestran claramente que en las cabras no es necesario un contacto permanente entre ambos sexos para lograr una reactivación eficiente de la actividad ovulatoria, si se utilizan machos sexualmente activos.

En este estudio, las proporciones de cabras que ovularon y las proporciones de cabras que presentaron ciclos ovulatorios cortos o normales no difirieron entre los grupos, independientemente de la duración del contacto con los machos. Nuestros datos están de acuerdo con los resultados reportados por Bedos *et al*. (2010, 2014), donde las cabras permanecieron en contacto con los machos durante 1, 2, 4 o 24 h por día durante 15 días. Por lo tanto, el resultado principal de nuestro estudio es que 15 minutos diarios de contacto con machos sexualmente activos fueron suficientes para inducir ovulaciones en la mayoría de las cabras. Los últimos resultados sugieren fuertemente que esta corta duración diaria del contacto con los machos fue suficiente para reactivar la secreción de GnRH/LH y, por lo tanto, permitir que se produzca la ovulación (Martínez-Alfaro *et al.,* 2014; Hawken y Martin, 2012).

Es probable que el éxito del contacto de corta duración entre machos y hembras para reactivar la actividad ovulatoria esté relacionado con el uso de machos sexualmente activos, y varios estudios están de acuerdo con esta hipótesis. Primero, los machos sexualmente activos indujeron a más hembras a ovular que los machos no tratados, que mostraron un comportamiento sexual débil (Delgadillo, 2011). Segundo, los machos sexualmente activos estimularon y mantuvieron una alta secreción de LH permitiendo que la ovulación ocurra en la mayoría de las hembras. Por el contrario, los machos sexualmente inactivos solo indujeron una elevación a corto plazo de la secreción de LH evitando que se produjera la ovulación en las hembras expuestas a ellas (Martínez-Alfaro *et al.,* 2014; Vielma *et al.,* 2009). En el presente estudio, los machos se volvieron sexualmente activos debido al tratamiento de días largos para aumentar sus concentraciones plasmáticas de testosterona, olor y comportamiento sexual durante la temporada no reproductiva, cuando se realizó el presente estudio. Por lo tanto, podemos concluir que el uso de machos sexualmente activos fue un elemento importante para reactivar la actividad ovulatoria de las cabras, incluso cuando el contacto diario se redujo a 15 minutos diarios.

Los resultados del Exp.1 en el estudio 2 demuestran que en nuestras condiciones experimentales, los machos en reposo sexual respondieron a la repentina exposición a las hembras inducidas artificialmente al estro al aumentar sus concentraciones plasmáticas de andrógenos, como ya se informó en otras razas y condiciones (Walkden-Brown *et al.,* 1994a; Gonzalez *et al.,* 1988; Schanbacher *et al.,* 1987). Por el contrario, los resultados de Exp. 2 no apoyaron nuestra hipótesis de que la presencia permanente de cabras inducidas al estro prevendría la variación estacional en las concentraciones de LH y andrógenos o las variaciones de la circunferencia escrotal. De hecho, la respuesta de los machos en interacción completa y continua con hembras en estro no difirió de la de los machos mantenidos con hembras en anestro o de los que se mantuvieron completamente aislados de las hembras. Es poco probable que esta falta de diferencia entre los grupos se deba a alguna incapacidad de los machos para responder a esas condiciones, ya que la respuesta a las hembras en celo se demostró claramente en el Exp. 1. Más bien, estos resultados indican que, contrariamente a nuestra hipótesis, el contacto completo continuo y las interacciones de comportamiento con las hembras sexualmente receptivas no previenen el efecto inhibitorio del fotoperiodo en los cambios estacionales asociados con la reproducción del macho.

Estos resultados contrastan con los resultados obtenidos en las hembras, en las que la presencia continua de machos sexualmente activos evita o reduce la acción inhibitoria del fotoperíodo sobre la estacionalidad reproductiva (Delgadillo *et al.,* 2015). Sin embargo, los resultados del Exp. 2 son congruentes con los de Giriboni *et al.* (2017), e indican que la falta de efecto de las hembras en el celo en el patrón general de la actividad reproductiva de los machos probablemente no fue el resultado de la falta de contacto total entre los sexos. Nuestras observaciones visuales directas durante el Exp. 2 sugieren que se produjeron relaciones sexuales intensas entre machos y hembras, especialmente en el grupo de machos que se encontraban en presencia continua de cabras de estro. Por lo tanto, es poco probable que la ausencia de un efecto de la presencia de cabras de estro en la actividad estacional de los machos se deba a la falta de interacciones sexuales entre los machos y hembras. En conjunto, estos hallazgos sugieren que el contacto físico total o parcial con cabras inducidas al estro no evita la inhibición estacional de la actividad del eje hipotálamo-pituitaria-gonadal en machos.

Los resultados del Exp. 2 indican que la presencia permanente de cabras inducidas al estro no mantiene de manera prolongada la secreción de LH y andrógenos. Esto no se debe a la falta de respuesta de los machos a las hembras en celo, ya que una respuesta a corto plazo de LH y andrógenos se registró en el Exp. 1. Estos resultados coinciden con los reportados anteriormente en caprinos y ovinos, en los cuales las concentraciones de LH y testosterona disminuyeron después de su aumento inicial, incluso en los machos que permanecieron en contacto con hembras en celo, (Gonzalez *et al.,* 1988; Sanford *et al.,* 1974). Es probable que el incremento de la testosterona observada al introducir las hembras en celo, haya actuado por retroacción negativa sobre la LH, disminuyendo su secreción. Esta disminución en la LH provocó a su vez, la reducción de la testosterona y del diámetro testicular

(Sanford *et al.,* 1974; Howland *et al.,* 1985; Walkden-Brown *et al.,* 1994c; Araki *et al.,* 2000).

En conclusión, la presencia permanente de hembras en celo no evita la reducción estacional de la LH, los andrógenos, y el diámetro testicular. Los machos parecen depender más estrictamente del fotoperiódo que de las interacciones socio-sexuales en el control de su estacionalidad reproductiva

CONCLUSIONES

Los resultados del primer estudio indican que la duración de contacto entre hembras y machos sexualmente activos puede reducirse de 1 hora a 15 minutos sin disminuir el porcentaje de hembras que ovulan.

Los resultados del segundo estudio indican que la presencia de hembras en estro no evitan la estacionalidad de las concentraciones plasmáticas de LH, andrógenos, y diámetro testicular.

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