UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSGRADO

LOS CAPRINOS SEXUALMENTE INEXPERTOS QUE PARTICIPAN EN EL EFECTO MACHO NO MUESTRAN RESPUESTA SEXUAL Y REPRODUCTIVA DIFERENTE A LOS CAPRINOS CON EXPERIENCIA SEXUAL PREVIA

Tesis

Que presenta JESSICA ANABEL LOYA CARRERA

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Tesis

Elaborada por JESSICA ANABEL LOYA CARRERA 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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AGRADECIMIENTOS

A la Universidad Autónoma Agraria Antonio Narro por las facilidades y recursos que me brindó para realizar mis estudios de Doctorado.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT), por el apoyo económico otorgado para llevar a cabo mis estudios de Doctorado (441658).

A mi asesora principal la Dra. Ilda Graciela Fernández García, por su confianza, apoyo incondicional hacia mi persona y por qué siempre creyó en mí persona.

A mi comité particular de asesoría: Dr. Horacio Hernández Hernández, Dr. Jesús Vielma Sifuentes y al Dr. Gerardo Duarte Moreno. Gracias por sus atinadas recomendaciones en este trabajo.

A mi asesora externa la Dra. Angélica María Terrazas García, muchísimas gracias por todo el apoyo brindado en la revisión de los artículos, sin duda usted fue un gran apoyo para poder alcanzar esta meta.

A mi compañeros de programa M.C. Edwin Mendieta y al Dr. Alfonso Muñoz, muchas gracias por todo el apoyo brindado durante estos tres años.

A todos los miembros del Centro de Investigación en Reproducción Caprina (CIRCA), que sin duda alguna contribuyeron en mi formación profesional y en mis estudios de doctorado.

A Esther Peña, Aurelia Nájera y Dolores López Magaña, por su invaluable apoyo administrativo durante mi etapa como estudiante de Doctorado.

A los caprinocultores el Sr. Andrés García y Sra. Imelda Rodríguez, por facilitarme las cabras y las instalaciones, lo que me permitió llevar a cabo el trabajo de campo. Al Sr. Jesús Palomo Reyna por la ayuda brindada durante la crianza de las hembras y machos utilizados en los experimentos.

A mis amigos M.C. Saraí Cueto, M.C. Edith Nandayapa, M.C. Antonio Castillo, por siempre estar en las buenas, en las malas y en las peores, por nunca dejarme sola y por convertirse en parte de mi familia. No tengo palabras para agradecerles todo el apoyo que me han brindado en estos tres años.

A mis amigos y compañeros de postgrado gracias por brindarme su amistad sincera, su apoyo, por sus buenas vibras y por ayudarme a confiar en mi misma. Gracias por ser parte de mi vida este último año convirtiéndolo en el mejor año del doctorado.

Y a todos esos maestros y amigos de la UAAAN que día a día me dieron su apoyo recordándome quien soy, a donde voy y hasta donde puedo llegar.

DEDICATORIAS

Quiero dedicar mi tesis a mi Familia, porque ellos han sido mi mayor motor en la vida y siempre han estado apoyándome en cada paso que doy:

A ti Mamá: Gracias por apoyarme siempre en todo lo que realizo, por apoyarme cuando decidí estudiar lejos de mi hogar, porque siempre me has apoyado en cada momento y decisión de mi vida ¡TE AMO MAMÁ!

A ti Papá: Gracias por ser siempre mi motor y el pilar que me mantiene fuerte, gracias por apoyarme y permitirme seguir mis sueños, por siempre brindarme una mano para apoyarme y un hombro para llorar cuando me he sentido derrotada ¡TE AMO PAPÁ!

A mis hermanas Selene, Jael y Zayra, y a mis 5 Sobrinos, por ser mis confidentes, mis paños de lágrimas y por ser mis mejores consejeros. Gracias por estar siempre a mi lado en las buenas y en las malas, por siempre estar para mí cuando las cosas van de maravilla y para limpiar mis lágrimas cuando las cosas no van bien.

Gracias sin duda porque sin usted no hubiera logrado esto, gracias por siempre recordarme quien soy y no dejarme vencer, gracias por enseñarme que las cosas buenas cuestan mucho cuando se consiguen de la manera correcta, pero se valoran más porque no cualquiera lo logra. Muchas gracias por siempre recordarme que puedo con esto y más.

¡LOS AMO!

Hay una fuerza motriz más poderosa que el vapor, la electricidad y la energía atómica: la voluntad

Albert Einstein

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RESUMEN

En algunas especies se ha demostrado que las hembras y machos sin experiencia sexual muestran bajo comportamiento sexual, y en consecuencia baja respuesta reproductiva. Estudio 1, se utilizaron tres grupos de cabras (n=9 cada uno): i) sin experiencia sexual, ii) con experiencia sexual completa, y iii) con experiencia sexual limitada. Tres machos cabríos fueron sometidos a un tratamiento fotoperiódico durante 2.5 meses (del 1 noviembre al 15 enero) de días largos para estimular su actividad sexual durante el reposo sexual natural (marzo-abril). Durante el anestro estacional, las hembras fueron expuestas a los machos foto-estimulados. Durante los tres primeros días post-introducción de los machos diariamente se registró la proceptividad y receptividad (20 min am y pm). Las hembras sin experiencia sexual y con experiencia sexual completa mostraron mayor frecuencia del abanicamiento de cola, que las hembras con experiencia sexual limitada ($P<0.001$). Las hembras sin experiencia sexual mostraron mayor olfateo hembra-hembra y emisión de orina, comparadas con los grupos con experiencia sexual completa y limitada ($P<0.001$ y $P<0.05$; respectivamente). Las hembras con experiencia sexual limitada mostraron mayor frecuencia montas de hembra-hembra que los grupos sin experiencia y con experiencia sexual completa ($P<0.05$). Además, las hembras con experiencia sexual completa mostraron mayor frecuencia de olfateo hembra-macho comparadas con las hembras sin experiencia y con experiencia sexual limitada ($P<0.001$). La receptividad no fue diferente entre los tres grupos de hembras ($P>0.05$). Se concluye que las cabras anéstricas sin experiencia sexual exhiben conductas proceptivas y receptividad de manera similar a las cabras con experiencia sexual expuestas a los machos foto-estimulados. Estudio 2, el objetivo fue para determinar si los machos sin experiencia sexual foto-estimulados exhiben comportamiento sexual durante el primer contacto con cabras nulíparas y multíparas, y estos machos pueden inducir la respuesta sexual y reproductiva en las cabras anéstricas. Se utilizaron machos cabríos sin experiencia sexual (n=6) foto-estimulados. En abril, dos grupos de hembras uno

de nulíparas y otro de multíparas (n=30/grupo) fueron puestas en contacto con los machos (3/grupo de hembras) durante 15 días consecutivos. El primer día (0) las aproximaciones no fueron diferentes entre los machos en contacto con las hembras nulíparas y multíparas. Mientras que en los días 1 y 2, la frecuencia en las aproximaciones fue mayor en los machos en contacto con las multíparas. En los días 0 y 1, la frecuencia de los olfateos ano-genitales no difirió entre los machos en contacto con las nulíparas y multíparas, mientras que en el día 2 fue mayor en los machos en contacto con las nulíparas. En cambio, en los días 0, 1 y 2, los intentos de monta, flehmen, auto-marcajes, y montas con y sin intromisión no difirieron entre los machos de ambos grupos. Las ovulaciones no difirieron entre nulíparas (96%) y multíparas (93%; P=1.00). La tasa de ovulación no difirió entre nulíparas (1.6 ± 0.1) y multíparas (1.4 ± 0.1 P=1.00). Los ciclos cortos (53% y 63%) y normales (43% y 30%) no presentaron diferencia entre ambos grupos. La tasa de preñez no difirió entre las cabras nulíparas (83%) y multíparas (80%). Se concluye que los machos sin experiencia sexual foto-estimulados son eficientes para inducir la actividad sexual y reproductiva en las cabras anéstricas nulíparas y multíparas.

PALABRAS CLAVE: Cabras, nulíparas, multíparas, fotoperiodo, efecto macho.

ABSTRACT

In some species it has been shown that females and males without sexual experience show under sexual behavior, and consequently low reproductive response. Study 1, three groups of goats (n = 9 each) were used: i) without sexual experience, ii) with complete sexual experience, and iii) with limited sexual experience. Three goats were subjected to photoperiodic treatment for 2.5 months (1 November-15 January) of long days to stimulate their sexual activity during natural sexual rest (March-April). During anestrous season, females were exposed to photo-stimulated males. During the first three days post-introduction of males daily was recorded the proceptivity and receptivity (20 min; am-pm). Sexually inexperienced females and those with complete sexual experience showed higher tail wagging than those with limited sexual experience ($P<0.001$). Sexually inexperienced females displayed higher female-female sniffing and emission of urine than those groups with complete and limited sexual experience ($P<0.001$ and $P<0.05$, respectively). Females with limited sexual experience displayed higher female-female mounts than those groups inexperienced and with complete sexual experience ($P<0.05$). In addition, females with complete sexual experience displayed higher male-female sniffing than those groups inexperienced and with limited sexual experience ($P<0.001$). Receptivity behavior did not differ between female groups ($P>0.05$). We concluded that sexually inexperienced anestrous females display proceptivity and receptivity behaviors as those sexually experienced exposed to photo-stimulated males. Study 2, the aim of this study was to determine whether sexually inexperienced photo-stimulated males display sexual behavior during

the first contact with nulliparous females, and whether males could induce sexual activity in these females. Sexually inexperienced males (n = 6) were used. Males were subjected to a treatment of long days to induce sexual activity during the sexual rest. In April, during anestrous season, two groups of nulliparous and multiparous (n = 30/group), females were placed in contact with males (3/group of females) for 15 consecutive days. On the first day, the nudging did not differ between males in contact with nulliparous and multiparous females. While on days 1 and 2, the nudging were higher in males in contact with multiparous than nulliparous. On days 0 and 1, the ano-genital sniffing did not differ between males in contact with the two groups of females, while on day 2 were higher in nulliparous. In contrast, on days 0, 1, and 2, the mounting attempts, flehmen, self-urination, and mounts with and without intromission did not differ be

tween males. Ovulations did not differ between nulliparous (96%) and multiparous (93%). Ovulation rate did not differ between nulliparous (1.6 ± 0.18) and multiparous (1.4 ± 0.75). Short (53% and 63%) and normal cycles (43% and 30%) did not differ between the two groups of females. Pregnancy rate did not differ between nulliparous (83%) and multiparous goats (80%). It is concluded that sexually inexperienced photo-stimulated males are efficient to induce sexual activity either in nulliparous or multiparous anestrous goats.

INTRODUCCIÓN

La caprinocultura es una actividad de gran importancia en la producción pecuaria de México con inventario nacional de 9 millones de cabezas de ganado. La Comarca Lagunera es una de las regiones del norte del país más importantes en la caprinocultura, esta región cuenta con alrededor del 4.8% del inventario caprino (SIAP, 2015). En la Comarca Lagunera (26 ^{o} N) los caprinos muestran estacionalidad reproductiva marcada, fenómeno controlado principalmente por el fotoperiodo (Delgadillo *et al.,* 1999). Las variaciones del fotoperiodo modulan la retroalimentación negativa que la testosterona y el estradiol ejerce sobre el eje hipotalámico-hipofisario-gonadal, constituyendo el mecanismo neuroendocrino responsable de la alternancia entre períodos de actividad y reposo sexual en ovinos y caprinos (Pelletier y Ortavant, 1975; Chemineau *et al.,* 1988; Delgadillo et al., 1999, Duarte *et al*., 2008). La estacionalidad sexual de los caprinos impide su reproducción de marzo–agosto (Delgadillo *et al.,* 2003), teniendo como consecuencia mayor disponibilidad de productos de origen caprino en los meses de diciembre–enero. En las hembras caprinas de las zonas templadas, los partos se concentran en diciembre y enero, lo que provoca que la producción de leche y carne sea mayor durante el invierno y en la primavera y sea menor durante el resto del año (Chemineau *et al.,* 2007). Para romper la estacionalidad reproductiva, es necesario tener conocimiento de diversas estrategias reproductivas, así como, de su aplicación en algunas de las razas caprinas y ovinas, con la finalidad de solucionar el problema que ocasiona la baja disponibilidad de productos de las especies antes mencionadas. En caprinos, se han utilizado diversas técnicas para estimular la actividad reproductiva como son los tratamientos hormonales en hembras y machos, el tratamiento con luz artificial en los machos y en ambos sexos la bioestimulación sexual, conocida como efecto macho. El efecto macho es la exposición de cabras anéstricas a machos sexualmente activos, aumentando la pulsatilidad de la secreción de LH, la conducta estral y la ovulación (Vielma *et al.,* 2009; Martínez-Alfaro *et al.,* 2014). Sin embargo, la

eficiencia del efecto macho depende de diversos factores como el tiempo de contacto, la novedad, familiaridad, experiencia, el número de partos, entre otros (Gelez *et al.,* 2004; Luna-Orozco *et al.,* 2008; Chanvallon *et al.,* 2010; Fernández *et al.,* 2011; Loya-Carrera *et al*., 2014; Muñoz *et al.,* 2016; Ramírez *et al.,* 2017).

En los caprinos del subtrópico mexicano la introducción de un macho cabrío sexualmente activo a un grupo de hembras anéstricas multíparas estimula la frecuencia y la amplitud de los pulsos de LH, el estro y la ovulación hasta en un 90% de las hembras expuestas al macho (Vielma *et al.,* 2009). En los machos con experiencia sexual, la actividad y comportamiento sexual pueden inducirse al someterse a 2.5 meses de días largos (16 horas/luz) a partir del 1 noviembre (Flores *et al.,* 2000; Delgadillo y Vélez, 2010). Este tratamiento permite inducir su actividad sexual durante la época de reposo sexual natural, es decir de febrero a abril (Delgadillo *et al.,* 2002; Rivas-Muñoz *et al.,* 2007).

Por ello, el presente estudio fue diseñado en dos experimentos para determinar si las hembras anéstricas sin experiencia sexual presentan comportamiento sexual al ser expuestas por primera vez a machos foto-estimulados, tal como ocurre en las hembras con experiencia sexual previa. Además determinar si los machos sin experiencia sexual foto-estimulados son capaces de inducir la respuesta sexual en cabras anéstricas nulíparas y multíparas durante el efecto macho.

REVISIÓN DE LITERATURA

Estacionalidad Reproductiva en los Caprinos de Zonas Subtropicales

La estacionalidad reproductiva es un mecanismo de adaptación desarrollado por algunos mamíferos silvestres como estrategia para minimizar el impacto negativo de un ambiente adverso en la supervivencia de las crías, es decir, que las crías nazcan cuando las condiciones del medio ambiente sean favorables para su supervivencia (Ortavant *et al.,* 1985; Bronson, 1989; Bronson y Heideman, 1994; Malpaux *et al.,* 1996; Arroyo, 2011). En los pequeños rumiantes como los caprinos y ovinos, la actividad sexual ocurre durante el otoño y el invierno, y los partos se producen a finales de invierno o principios de primavera (caprinos: Ortavant *et al.,* 1985; ovinos: Gerlach y Aurich, 2000).

La estacionalidad sexual se caracteriza por cambios a nivel endocrino, de comportamiento, gonadal y anatómico, y está sincronizada por factores del medio ambiente, entre otros, como el fotoperiodo y las relaciones socio-sexuales (Malpaux *et al.,* 1996; Arroyo, 2011).

En las zonas subtropicales (23 a 40º Norte o Sur) existen razas de cabras y ovejas que manifiestan estacionalidad reproductiva, es decir, que tanto los machos como las hembras expresan durante el año un período de actividad sexual y otro de reposo sexual (Restall, 1992; Walkden-Brown *et al.,* 1994; Delgadillo *et al.,* 1999).

Hembras

Las hembras originarias de zonas subtropicales presentan marcada estacionalidad reproductiva (Delgadillo, 2011). En las hembras de la raza malagueña (36º N), y las criollas de la Comarca Lagunera (26º N) la estación sexual inicia en agosto-septiembre (verano) y termina en febrero-marzo (invierno-primavera; Gómez-Brunet *et al.,* 2003; Duarte *et al.,* 2008; Figura 1).

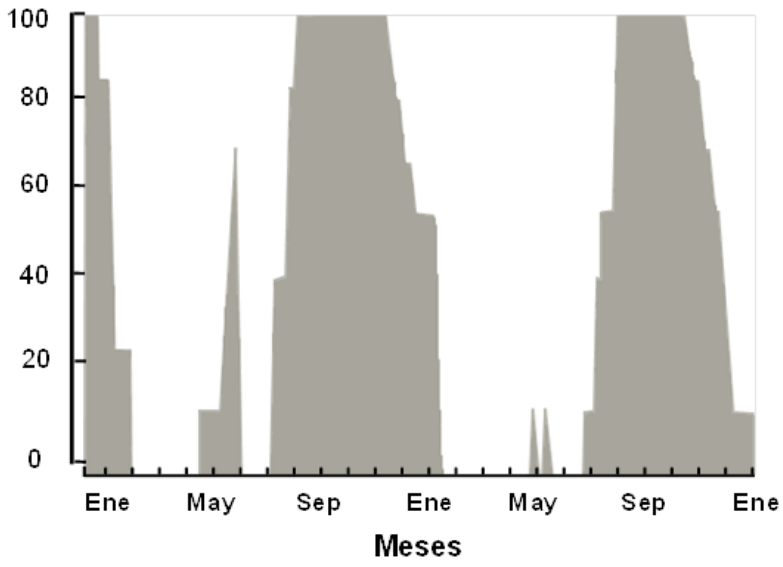

Figura 1.Variaciones estacionales de la actividad ovulatoria en las cabras locales del norte de México (26° N) mantenidas en estabulación, alimentadas adecuadamente y sometidas a las variaciones naturales del fotoperíodo (Adaptada de Duarte *et al.,* 2008).

La mayoría de las hembras de las razas caprinas originarias de latitudes templadas (>40º) presentan estacionalidad reproductiva (Amoah *et al.,* 1996); por ejemplo, las razas Alpina y Saanen, que tienen su actividad sexual de septiembre (otoño) a febrero (invierno), presentan anestro de marzo (primavera) a agosto (verano; Chemineau *et al.,* 1992).

Contrariamente, las hembras criollas originarias de zonas tropicales como aquellas de la Isla de Guadalupe en el Caribe, tienen la capacidad de reproducirse durante todo el año, ya que no presentan una estacionalidad reproductiva (Chemineau, 1993).

Ciclos estral y ovulatorio en cabras

Las cabras y ovejas que manifiestan estacionalidad reproductiva, presentan varios ciclos estrales y ovulatorios durante la estación sexual en caso de quedar gestantes, por esta razón se definen como poliéstricas estacionales. El

ciclo estral comprende un conjunto de cambios endócrinos, morfológicos y conductuales que conllevan a la expresión del estro (celo), la ovulación y la preparación del tracto genital para la copulación, fertilización e implantación embrionaria. En las cabras, el ciclo estral, se define como el intervalo que existe entre 2 expresiones sucesivas del comportamiento de estro. Dicho ciclo estral tienen una duración promedio de 21 días (rango 17-25 días; Chemineau *et al.,* 1992). Sin embargo, existen también ciclos estrales de corta (<17 días) y larga duración (>25 días; Chemineau *et al.,* 1992). Los ciclos cortos aparecen generalmente al inicio de la estación sexual, al final del anestro postparto o cuando las hembras son sometidas al efecto macho (Chemineau, 1983; Flores *et al.,* 2000). En cambio, los ciclos largos son más frecuentes al final de la estación reproductiva (Chemineau *et al.,* 1992).

El ciclo estral en la cabra se divide en dos fases: folicular y lútea (Driancourt, 2001; Rosa y Bryant, 2003). La fase folicular se subdivide en dos etapas, proestro y estro. El proestro tiene una duración promedio de 3 días, partiendo de la regresión del cuerpo lúteo; esta etapa se caracteriza por una disminución en la secreción de progesterona, y por un rápido crecimiento folicular e incremento en la secreción de estradiol (Medan *et al.,* 2003; Bartlewski *et al.,* 2011). Durante el proestro (dominancia folicular), la FSH secretada por la adenohipófisis estimula el crecimiento folicular favoreciendo la selección de 2 o 3 folículos con diámetro >4 mm que continúan su crecimiento hasta la ovulación. Los folículos restantes presentan atresia folicular (Driancourt, 2001; Medan *et al.,* 2003; Fatet *et al.,* 2011). El estro es provocado por las altas concentraciones de estradiol secretado por los folículos, en este periodo se presenta la receptividad sexual, y tiene una duración de 24-36 h (Shelton, 1978; Camp *et al.,* 1983; Rosa y Bryant, 2003; Fatet *et al.,* 2011).Después de 30 a 36 horas de iniciado el estro se produce la ovulación, a causa de la retroalimentación positiva (feedback positivo) del estradiol sobre el eje hipotálamo-hipófisis, incrementando la secreción de la hormona liberadora de gonadotropinas (GnRH), que a su vez induce el pico pre-ovulatorio de LH (Akusu *et al.,* 1986; Bartlewski *et al.,* 2011).

La fase lútea se subdivide en metaestro y diestro (Chemineau *et al.,* 1992; Fatet *et al.,* 2011). El metaestro es la etapa siguiente a la ovulación, durante la cual se ve disminuida la secreción de estradiol y tiene una duración de 2-5 días. Durante esta etapa, las células de la granulosa y teca interna del folículo que ovuló, inician su luteinización bajo la influencia de la LH (Fatet *et al.,* 2011). El diestro se caracteriza por la presencia del cuerpo lúteo funcional y tiene una duración promedio de 12 días (Medan *et al.,* 2003). La progesterona secretada por el cuerpo lúteo ejerce una retroalimentación negativa sobre el GnRH y las gonadotropinas LH y FSH, por lo que sus concentraciones plasmáticas son bajas durante el diestro. En caso de que ocurra la fecundación, el cuerpo lúteo persiste manteniendo elevadas las concentraciones plasmáticas de progesterona y por lo tanto la gestación; en rumiantes la gestación se mantiene por medio de la señal que ejerce el embrión mediante el interferón TAU. En caso de no existir fecundación, el cuerpo lúteo es destruido por acción de la prostaglandina F2α secretada por las células endometriales del útero, provocando la disminución de las concentraciones plasmáticas de progesterona, y el fin de la retroalimentación negativa sobre el eje hipotálamo-hipófisis, permitiendo el inicio de un nuevo ciclo estral (Fatet *et al*., 2011).

Macho

Al igual que en las hembras, los machos cabríos presentan estacionalidad reproductiva. En regiones subtropicales, las razas de ovinos y caprinos muestran inactividad sexual, por ejemplo los machos criollos de la Comarca Lagunera y la raza Payoya en el sur de España (37° N), inician la estación sexual en mayo-junio y termina en diciembre-enero, presentando un periodo de reposo sexual que inicia en enero-febrero y termina en abril-mayo (Delgadillo *et al.,* 1999, 2002; Zarazaga *et al.,* 2009).

Durante la estación de actividad sexual se presentan diversos cambios fisiológicos en dichos machos, entre ellos el aumento de la talla testicular, en las concentraciones plasmáticas de testosterona, del olor y del

comportamiento sexual (Walkden-Brown *et al.,* 1997; Pérez-Clariget *et al.,* 1998; Delgadillo *et al.,* 1999).

Fotoperiodo

En los caprinos originarios de latitudes templadas y subtropicales, el fotoperiodo es el principal factor medio ambiental que determina la estacionalidad de la actividad sexual (Malpaux *et al.,* 1996; Martin *et al.,* 1999; Delgadillo *et al.,* 2004; Duarte *et al.,* 2010). En el subtrópico los caprinos mantenidos bajo las variaciones naturales de luz, inician la actividad sexual durante los días más largos y terminan en los días más cortos del año (Walkden-Brown *et al.,* 1994; Delgadillo *et al.,* 1999, 2002; Duarte *et al.,* 2008).

Esta información de la variación luminosa física (fotón) es captada inicialmente en el ojo por la retina, en la cual se encuentran células ganglionares que expresan melanopsinas en forma de un fotopigmento y regula la función del núcleo supraquiasmático el cual transmite el impulso por vía nerviosa hasta la glándula pineal, la que produce melatonina en respuesta a la percepción de luz y de la oscuridad (Malpaux *et al.,* 1997). La secreción de esta hormona es de tipo endógena y depende de la duración del periodo de obscuridad (Malpaux *et al.,* 1987, 1988; Delgadillo y Chemineau, 1992; Delgadillo *et al.,* 2001). El perfil de la secreción de la melatonina en pequeños rumiantes durante los días cortos, actúan en el hipotálamo premamilar para estimular la secreción de GnRH y la LH y FSH en la hipófisis, por consecuencia estimula la actividad sexual en machos y hembras (Malpaux *et al.,* 1999).

Los días largos sincronizan probablemente el inicio de la estación sexual, mientras que los días cortos determinan la duración de la actividad sexual (Malpaux *et al.,* 1989, Bustos y Torres-Díaz, 2012).

Bioestimulación Sexual

En los pequeños rumiantes existen diversos métodos de inducción de la actividad sexual, entre ellos, destaca la bioestimulación sexual conocido como "efecto macho". Dicho método consiste en inducir la actividad ovulatoria en las hembras anéstricas mediante la introducción de un macho en su corral (Underwood, 1944; Shelton, 1960; Delgadillo *et al.,* 2015).

Durante el efecto macho se ejerce un estímulo, el cual provoca un incremento de la pulsatilidad de LH, sincronizando el estro y la ovulación (Flores *et al.,* 2000; Delgadillo *et al.,* 2004; Martin *et al.,* 2004; Ungerfeld *et al.,* 2004). Esto debido a que inmediatamente después de la introducción del macho en un grupo de hembras anéstricas se estimula en ellas la secreción de LH, pasando de 0.3 pulsos a 2.2 pulsos en las primeras 3 horas de contacto macho-hembra. La secreción de LH permanece elevada mientras exista el contacto entre los sexos (Vielma *et al.,* 2009), la cual originará un pico preovulatorio ocasionando la ovulación en los primeros 5 días posteriores al primer contacto entre el macho y las hembras.

Sin embargo, en caprinos durante el efecto macho es común que se presente ciclos ovulatorios de duración corta (17 días), y las ovulaciones pueden disociarse de los estros. En la mayoría de los casos, las hembras vuelven a

ovular en un periodo de 5 a 7 días después de la primera ovulación. Esta segunda ovulación es acompañada de conducta estral en la mayoría de los casos, y el ciclo ovulatorio es de duración normal (Chemineau *et al.,* 1983, 1987; Walkden-Brown *et al.,* 1999; Figura 2).

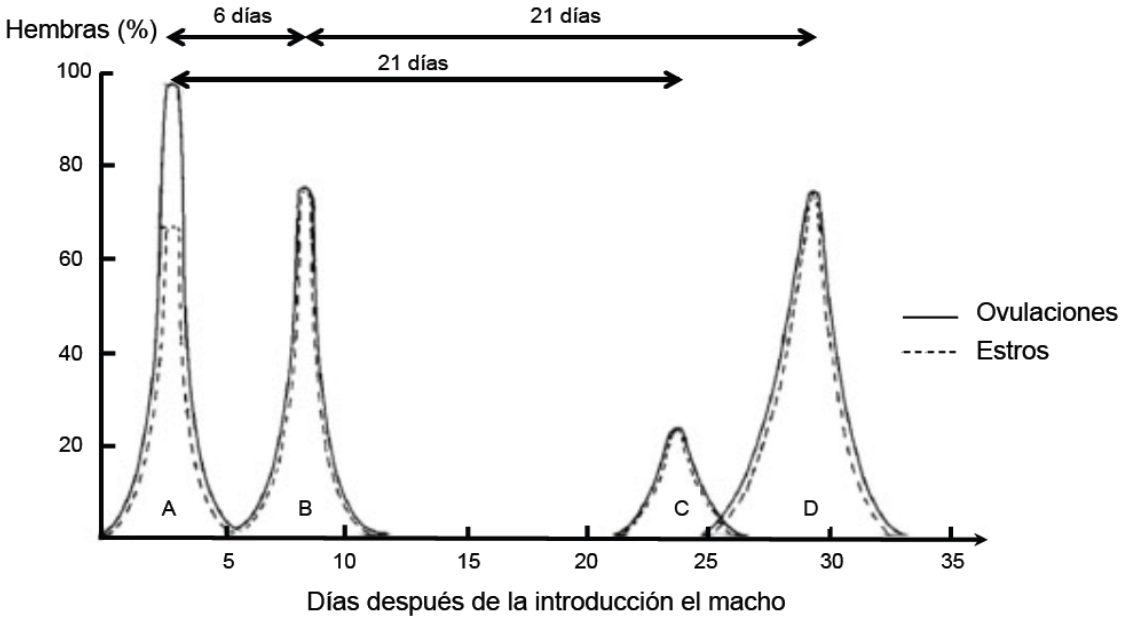

Figura 2. Representación esquemática de la respuesta al efecto macho en cabras anovulatorias. Más del 90 % de las hembras ovulan alrededor del día 3 post-introducción de los machos (pico A). Esta primera ovulación está asociada con un comportamiento de estro (60%). A partir del día 3 la mayoría de las hembras que ovularon experimentaron un ciclo ovárico corto y ovularon nuevamente 6 días después (pico B). Si no se presenta la gestación, las hembras ovulan por tercera ocasión 21 días más tarde (pico D). El 25 % de las cabras experimentan un ciclo normal después de la primera ovulación y, si no quedan gestantes, ovulan nuevamente 21 días después (pico C). Las ovulaciones de los picos B, C y D están asociados con comportamiento de estro (Chemineau, 1987).

Comportamiento Sexual en la Hembra

En el comportamiento sexual de las hembras mamíferas se pueden distinguir 3 características: la atractividad, la proceptividad y la receptividad (Beach,

1976). En la atractividad la hembra ovina o caprina, tiene cambios fisiológicos y conductuales pasivos que influyen en el macho. La atractividad se determina mediante el comportamiento sexual del macho, esto es, cuando observa, se aproxima y olfatea a la hembra (Beach, 1976; Gonyou, 1991; Roselli y Stormshak. 2010), en ovinos, como en los caprinos, la atractividad está influenciada por los niveles hormonales y es más intensa cuándo se incrementan las concentraciones de estradiol en las hembras, antes de la ovulación (Fabre-Nys y Gelez, 2007).

La proceptividad son los comportamientos sexuales exhibidos por la hembra, dirigidos al macho con el fin de iniciar e inducir la relación o el contacto sexual. Para diferenciar la atractividad de la proceptividad se considera que la atractividad sólo incluye los estímulos no-conductuales, mientras que en la proceptividad la hembra ya realiza comportamientos exclusivos para atraer al macho (Fabre-Nys y Gelez, 2007). En las ovejas dentro de estas conductas proceptivas podemos encontrar el movimiento de cola, la monta hembra-hembra, el olfateo hembra-hembra, el olfateo hembra-macho, entre otros (Gelez *et al.,* 2004)

La receptividad es la fase en la que la hembra permite la monta y/o cópula, por lo que en esta fase se incluye la expresión de todos los comportamientos que facilitan la cópula, mediante conductas pre-copulatorias. Una hembra sexualmente receptiva asume una postura que facilita la intromisión y eyaculación en la vagina, lo cual se conoce como inmovilidad activa, seguido de la monta y la cópula (Beach, 1976). En el caso de ovinos las hembras

permanecen quietas y levantan la cola para facilitar la penetración (Gelez *et al.,* 2004).

Factores que Modifican la Respuesta Sexual en las Hembras

Experiencia sexual

Para fines del presente estudio se considera a una hembra sin experiencia sexual, como aquella que no ha tenido contacto sexual desde los 3 días de edad, es decir que no ha percibido alguna señal sensorial emitida por un macho de su misma especie (auditiva, olfativa, visual y táctil; Gelez *et al.,* 2004; Hawken *et al.,* 2008).

En ovejas, se ha demostrado que la experiencia sexual influye en los comportamientos sexuales de proceptividad y receptividad, así como, en la respuesta reproductiva (Gelez *et al.,* 2004; Hawken *et al.,* 2008). Además, se ha demostrado que las hembras ovinas y caprinas adquieren experiencia sexual mediante las relaciones socio-sexuales, es decir, mediante el cortejo, la monta con intromisión, por la percepción del sexo opuesto cuando el macho se encuentra sexualmente activo o las hembras se encuentran receptivas (Gelez *et al.,* 2004; Fernández *et al.,* 2011).

Murtagh *et al.* (1984) observaron que las ovejas (Merino) sin experiencia sexual previa exhiben un comportamiento sexual bajo, además de baja respuesta reproductiva. En el estudio antes mencionado, se encontró que las ovejas sin experiencia sexual presentan menos ovulaciones (27%), en comparación con aquellas con experiencia sexual (74%). Además, está reportado que en ovinos, cuando se exponen a los machos durante la época

de reproducción natural, el 62% de las ovejas sin experiencia sexual presentan conducta estral, mientras que el 100% de las ovejas con experiencia sexual lo hacen (Gelez *et al.,* 2004). También se ha demostrado, que las ovejas sin experiencia sexual son menos proceptivas y receptivas en comparación con las que tienen experiencia sexual (Katz y McDonald, 1992; Shearer y Katz, 2006; Haulenbeek y Katz, 2011).

En caprinos originarios de la Comarca Lagunera, en las hembras la presencia de estros no difiere entre aquellas con experiencia y sin experiencia sexual, cuando son expuestas a machos foto-estimulados (95% y 100%, respectivamente; Fernández *et al.,* 2011).

Número de partos

Está demostrado que el número de partos que ha experimentado una hembra influyen en la respuesta sexual. Por ejemplo, Murthagh *et al.* (1984) y Walkden-Brown *et al.* (1993), demostraron que la respuesta sexual, determinada por el comportamiento estral y la actividad ovulatoria en ovejas y cabras, es menor en nulíparas (hembras que no han tenido partos) comparadas con las hembras multíparas (hembras que han experimentado más de un parto).

Además, las ovejas nulíparas (Merino) presentan menos ovulaciones, así como, menos ciclos ovulatorios que las multíparas (42% y 100% respectivamente; Chanvallon *et al.,* 2010). En las cabras (Serrana), las multíparas presentan mayor frecuencia de 2 o 3 cuerpos lúteos, a diferencia de las nulíparas (76% y 18% respectivamente; Simões *et al.,* 2008). También,

en cabras criollas de Venezuela nulíparas se observó mayor número de ciclos estrales cortos que en multíparas (27% y 15% respectivamente; González-Stagnaro y Madrid-Bury 1993). Los estudios mencionados sugieren que tanto las ovejas como las cabras nulíparas, muestran baja respuesta sexual, en comparación a las multíparas.

Sin embargo, Luna-Orozco *et al.* (2008) demostraron que el número de partos no modifica la respuesta sexual, cuando las cabras anéstricas son expuestas a machos foto-estimulados durante el anestro estacional. En este estudio, se reportó que las cabras nulíparas o multíparas exhiben respuesta estral y ovulatoria similar (100% y 100%, estro; 100% y 95%, ovulación).

Tiempo de contacto entre machos y hembras

El tiempo de contacto entre sexos es otra variable que puede modificar la respuesta estral y ovulatoria en las hembras expuestas al efecto macho. Puede reducirse la duración del contacto entre machos foto-estimulados y hembras anéstricas sin verse afectado el porcentaje de cabras que ovulan durante este proceso (Bedos *et al.,* 2010, 2012, 2014).

Ponce *et al.* (2015), demostraron que 10, 5 y hasta con 1 día de contacto entre ambos sexos es suficiente para que las hembras presenten ovulación.

Se ha demostrado que el tiempo de contacto directo entre los dos sexos tan corto como de 15 minutos por día durante 15 días consecutivos fue suficiente para inducir la ovulación en las cabras anéstricas (Ramírez *et al.,* 2017). Estos resultados indican que el tiempo de contacto entre los dos sexos puede

reducirse sin disminuir la proporción de cabras que ovulan, si las cabras son expuestas a machos cabríos foto-estimulados.

Comportamiento sexual en el macho

En el macho cabrío, la conducta sexual es expresada por conductas características como son: las aproximaciones laterales, el olfateo ano-genital, el intento de monta, el automarcaje, el flehmen y las montas con intromisión (Flores *et al.,* 2000; Fabre-Nys, 2000; Figura 3). Los machos foto-estimulados presentan comportamiento sexual con una cantidad y calidad similar al mostrado por los machos que se encuentran la estación de actividad sexual natural. En consecuencia, los machos foto-estimulados son eficientes para estimular la actividad sexual en las hembras sometidas al efecto macho durante el anestro estacional (Bedos *et al.,* 2016).

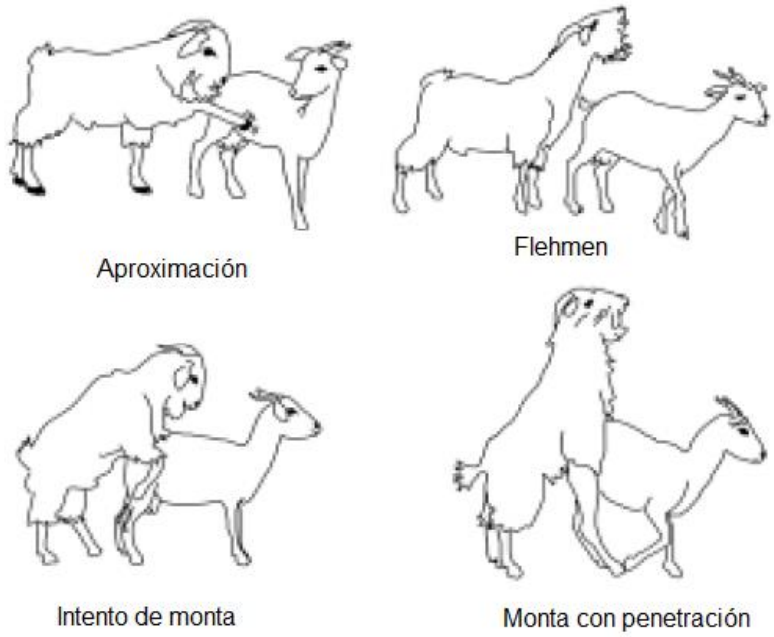

Figura 3. Comportamiento sexual de los machos caprinos (adaptado de Fabre-Nys, 2000).

Intensidad del comportamiento sexual en el macho

En ovejas y cabras se demostró que la intensidad del comportamiento sexual de los machos es un elemento clave para inducir el estro y la ovulación, en la mayoría de las hembras expuestas a los machos (Perkins y Fitzgerald, 1994; Flores *et al.,* 2000; Delgadillo *et al.,* 2002). En su trabajo Perkins y Fitzgerald (1994), encontraron mayor porcentaje de ovejas que ovularon cuando fueron expuestas a machos que mostraron alto comportamiento sexual, comparadas con aquellas expuestas a machos con bajo comportamiento sexual (95% y 78%, respectivamente). Posteriormente, Flores *et al*. (2000), expusieron machos sexualmente inactivos (mantenidos en condiciones de fotoperiodo natural) y machos sexualmente activos (sometidos a un tratamiento de foto-periodo de días largos por 2.5 meses más melatonina) a hembras anéstricas los resultados indicaron que un bajo porcentaje de cabras ovularon al ser expuestas a los machos sexualmente inactivos, en cambio la totalidad de las cabras mostraron ovulaciones cuando fueron expuestas a machos sexualmente activos (6% y 100%, respectivamente).

Delgadillo *et al.* (2015) expusieron a hembras caprinas a machos sexualmente activos, durante un periodo de 16 meses (a partir de enero), dichas hembras mantuvieron la actividad cíclica ovárica sin importar que éstas se encontraban bajo la variación natural del fotoperiodo. En dicho estudio se demostró, mediante la determinación de progesterona plasmática, que todas las hembras que permanecieron en contacto con machos sexualmente activos mostraron ciclicidad ovárica, esto sucedió durante dos estaciones de anestro

consecutivas, mientras que las hembras expuestas a machos inactivos, no mostraron tal ciclidad ovárica durante la época de anestro estacional.

Factores que Modifican la Respuesta Sexual en Machos Cabríos

Tratamiento de luz artificial adicional

La inducción de la actividad sexual de los machos durante los meses de reposo, puede llevarse a cabo mediante la aplicación de un tratamiento de fotoperiodo de días largos (Delgadillo *et al.,* 2002). En los machos cabríos de la Comarca Lagunera, la actividad sexual se puede estimular al manipular el fotoperiodo combinando días largos artificiales con fotoperiodo natural. Esto implica que los machos son expuestos a 2.5 meses de días largos (16 h luz/día), a partir del 1 de noviembre, al término del tratamiento los machos percibieron días cortos crecientes. Este tratamiento estimula la secreción de testosterona, la motivación sexual, el olor y las vocalizaciones de los machos en marzo y abril, meses los cuales corresponden al periodo de reposo sexual (Rivas-Muñoz *et al.,* 2007; Delgadillo *et al.,* 2002, 2012; Bedos *et al.,* 2012). Sin embargo, es posible acortar la duración del tratamiento fotoperiódico, Ponce *et al*. (2014), demostraron que con la aplicación de 1.5 meses de días largos a partir del 1 de diciembre, seguidos del fotoperiodo natural, en marzo y abril se estimula la secreción de testosterona y el comportamiento sexual en los machos cabríos de la misma manera que en aquellos que fueron sometidos a 2.5 meses de días largos. Estás técnicas de inducción en la motivación sexual en los machos cabríos, durante el reposo sexual son amigables con el medio ambiente, de bajo costo y fáciles de implementar en

los sistemas de producción intensivos o extensivos, que se encuentran generalmente en las latitudes subtropicales. Además, esta técnica es sustentable por no requerir del uso de hormonas exógenas (Delgadillo, 2011).

Experiencia sexual en machos

Por lo general, los machos de algunas especies de mamíferos domésticos muestran bajo comportamiento sexual cuando se exponen por primera vez a las hembras (Katz *et al.,* 1988). Por ejemplo, Hulet *et al.* (1964), observaron que al exponer carneros, por primera vez, a hembras en estro fueron sexualmente inactivos.

También está reportado que cuando se crían y se desarrollan carneros en un solo grupo unisexual, al llegar a la edad adulta y ser expuestos a ovejas, estos machos muestran un bajo desempeño sexual, además muestran menor tasa de eyaculación (Price *et al.,* 1994). Asimismo, cuando se exponen carneros jóvenes sin experiencia sexual, por primera vez a ovejas en estro, dichos carneros muestran baja motivación sexual (20-35%, Katz *et al.,* 1988).

En caprinos, los machos sin experiencia sexual, criados en grupo unisexual y sometidos a un tratamiento fotoperiódico de días largos, muestran comportamiento sexual cuando son puestos en contacto primera vez con cabras anovulatorias. Los machos sin experiencia sexual estimulan la actividad reproductiva en las hembras de manera similar a los machos cabríos con experiencia sexual (Flores Medina, 2011; Bedos *et al.,* 2014). Estos estudios sugieren que el comportamiento sexual exhibido por el macho es un elemento clave en la estimulación sexual en las cabras en anestro estacional.

HIPÓTESIS

Estudio 1. Las cabras anéstricas sin experiencia sexual pueden mostrar baja motivación sexual, comparadas con aquellas con experiencia sexual previa, cuando son expuestas por primera vez a machos foto-estimulados durante el anestro estacional.

Estudio 2. Los machos cabríos sin experiencia sexual y foto-estimulados, son capaces de mostrar buena motivación sexual durante el primer contacto con hembras, asimismo, dicha respuesta no es afectada por el número de partos de las hembras.

OBJETIVOS

Estudio 1. Determinar si las hembras anéstricas, sin experiencia sexual previa, en su primera exposición a machos sexualmente activos, si son capaces de mostrar conductas proceptivas y receptivas, como aquellas con experiencia sexual.

Estudio 2. Determinar si los machos sin experiencia sexual y foto-estimulados muestran motivación sexual durante el primer contacto con cabras anéstricas, y evaluar si esta respuesta varía de acuerdo al número de partos de las hembras.

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ARTÍCULO 1

J Anim Behav Biometeorol (2017) 5:64-71

ORIGINAL ARTICLE

Sexually inexperienced anestrous goats are able to exhibit sexual behaviours exposed to sexually active bucks

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Received: December 28, 2016 ▪ Revised: February 02, 2017 ▪ Accepted: February 20, 2017

Abstract The aim of this study was to determine whether sexually inexperienced females could display proceptivity and receptivity behaviours as the experienced, in the first exposure to males. Three groups of females (n=9 each) were used: i) sexually inexperienced, ii) with complete sexual experience, and iii) with limited sexual experience. Three male goats were subjected to photoperiodic treatment for 2.5 months of long days to stimulate their sexual activity during the natural sexual rest (March-April). During anestrous season, females were exposed to photo-stimulated males. Sexual behaviours were recorded during the first three days post-introduction of the males into female groups, in two daily periods of 20 min each. Sexually inexperienced females and those with complete sexual experience showed higher tail wagging than those with limited sexual experience ($P<0.001$). Sexually inexperienced females displayed higher female-female sniffing and emission of urine than those groups with complete and limited sexual experience ($P<0.001$ and $P<0.05$, respectively). Females with limited sexual experience displayed higher female-female mounts than those groups inexperienced and with complete sexual experience ($P<0.05$). In addition, females with complete sexual experience displayed higher female-male sniffing than those groups inexperienced and with limited sexual experience ($P<0.001$). Receptivity behaviour did not differ between female groups ($P>0.05$). We concluded that sexually inexperienced anestrous females display proceptivity and receptivity behaviours as those sexually experienced exposed to photo-stimulated males.

Keywords: anovulatory does, *Capra hircus*, male effect, photoperiod, seasonality

Introduction

Sexual activity in sheep and goat females is regulated by photoperiod (Duarte et al 2008; Abecia et al 2015). In these females the period of sexual activity is known as natural reproductive season, and another one of sexual inactivity is called seasonal anestrous (Delgadillo et al 1991, 1992; Restall 1992; Chemineau et al 2006; Duarte et al 2008). If sheep and goat females are exposed suddenly to a male during seasonal anestrous, in a relatively short time they exhibit sexual behaviours. This technique of sexual biostimulation is known as the male effect (Shelton 1960; Martin et al 1986; Chemineau 1987; Delgadillo et al 2009).

In the mammals, it is known that the females in sexual activity display stereotyped behaviours towards the male that are expressed in two motivational phases, and are known as proceptivity and receptivity (Beach 1976). During the proceptive phase, females display behaviours in order to attract the attention of the male, and initiate sexual contact with him; while in the receptive phase, females allow the consummation of the sexual encounter with the male (acceptance of mounts with intromission; Beach 1976; Price 1985; Fabre-Nys and Gelez 2007). For example, sexually active sow exhibits proceptivity behaviours when is in physical contact with the boar, in addition they display head-butting, and sniffs the flanks and ano-genital region of the boar (Hemsworth 1985). In comparison, in ewes during the same phase, they exhibit movements with the head towards to the male, remain close to him, and exhibit tail wagging (Gonyou 1991; Gelez and Fabre-Nys 2004; Gelez et al 2004). In sheep, sexually inexperienced females, i.e. those who have not had socio-sexual contact with males are less proceptive and receptive than those with sexual experience (Gelez et al 2004; Hawken et al 2008). In comparison, sexually experienced goats, i.e. when females have had socio-sexual contact with males, during natural or induced estrus, they exhibit proceptivity behaviours such as tail wagging and female-female mounts (Llewelyn et al 1993;

DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

Imwalle and Katz 2004). In fact, sexually experienced anestrous female goats are more receptive when exposed to photo-stimulated males than non-photo-stimulated (Delgadillo et al 2002; Rivas-Muñoz et al 2007; Loya-Carrera et al 2014; Muñoz et al 2016).

To our knowledge, there are no studies in goats which have reported neither the effect of lack of sexual experience in females, nor the restriction of sexual experience, to display of proceptivity behaviours during seasonal anestrous when exposed to photo-stimulated males. Therefore, the hypothesis in the present study states that sexually inexperienced goats, during anestrous season, could display lower sexual behaviours in their first exposure to males, than sexually experienced goats. The objective of the present study was to determine whether sexually inexperienced females, in their first exposure to photo-stimulated males, could exhibit sexual behaviours of proceptivity and receptivity as those sexually experienced goats.

Materials and Methods

Ethical note

The management of the females and males in the present study was carried out following the protocol of the Norma Oficial Mexicana, according to specifications for the production, care and management of laboratory animals (SAGARPA 2001). The early separation of the kids from their mothers, and artificial breastfeeding that they received did not disrupt normal growth and development in these females.

Animals and description of experimental groups

Twenty-seven creole goat kids were born on January 10 ± 2 days (mean value ± standard error of mean) from the Laguna region in the State of Coahuila (latitude 26°23'N and longitude 104°47'W) during the natural breeding season were used. The goat kids were kept at all times in housing. The goat kids were separated from their mothers at 3 days of age, and were fed with goat´s milk through an artificial breastfeeding system, which has shown have not negative effects on the development and weaning of goat kids (Lu and Potchoiba 1988; Luo et al 2000). In addition, were given forage and water *ad libitum*. The goat kids remained in these conditions until 40 days of age. Then, females were randomly distributed into three groups. The first, sexually inexperienced females (n= 9) were kept isolated from males in a pen of 5 x 5 m. The second, with complete sexual experienced females (n= 9) were maintained in complete permanent contact with two vasectomized males. These females and males were placed in a single pen (5 × 5 m). The third, with limited sexual experienced females (n= 9) was maintained in restricted permanent contact with two vasectomized males. Females were placed in a pen (5 × 5 m) adjacent to vasectomized males (5 × 2 m), separated by a division of wire-mesh. Vasectomized males were 2 years old on average. Females with complete and limited sexual experience remained in contact with vasectomized males since artificial breastfeeding was suspended (40 days old), until the next natural breeding season when females were 12 months old. Afterwards, the vasectomized males were removed from the pens. Females remained in these conditions until behavioural measurements were carried out.

Females were fed with alfalfa hay *ad libitum* (18% crude protein, 1.95 Mcal/kg) and commercial concentrate (18% crude protein; 2.05 Mcal/kg) according to their nutritional requirements since weaning until the end of the study. Vasectomized males were fed with alfalfa hay *ad libitum* (18% crude protein; 1.95 Mcal/kg) and 300 g of commercial concentrate (14% crude protein, 2.5 Mcal/kg). For females and males drinking water and mineral salts (12% phosphorus and 11% calcium) were always available.

Treatment of artificial photoperiod applied to males

In order to induce an increase in sexual behaviour, odor, vocalizations and sperm production in males during the natural sexual rest (March-April; Delgadillo et al 2002), a treatment of artificial photoperiod was applied. Entire males (n= 3) of 3 years of age on average were used. Males were housed in outdoor pen (5 × 5 m) in order to receive the treatment of artificial photoperiod (with lamps of 65 W). Light intensity was at least 300 lx at the level of the eyes of the males (Figure 1). The treatment of long days applied to the males was for 2.5 months (16 h of light/day), starting on November 1 to January 15. From January 16 the males perceived the natural photoperiod.

Preparation of females

In goats of this region, the seasonal anestrous is from March to September (Duarte et al 2008). Therefore, in the month of March, when females were 14 months old, they were submitted to an evaluation to determine their anovulatory status. The anovulation was determined with the help of studies of ultrasonography, and was resolved when corpora lutea were not detected. The ultrasonography studies were performed on March 10 and 20 before introducing photo-stimulated males into three groups of females. The ultrasound used was an Aloka SSD-500 (Tokyo, Japan) with a 7.5 MHz transducer. In addition, three days before beginning the experiment, and based on the scale described by Walkden-Brown et al (1997), body condition score was measured in sexually experienced females, with complete

DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

and limited sexual experience (2.6 ± 0.08, 2.5 ± 0.09 and 2.5 ± 0.07, respectively; P>0.05).

Figure 1 Males were submitted to a treatment of artificial long days, light intensity was at least 300 lx at the level lateral of the eyes of the males. The treatment of artificial long days applied to the males was for 2.5 months (16 h of light/day) from November 1 to January 15.

Male effect

On March 25 (day 0; 08:00 h), a photo-stimulated male was placed in contact with each group of females (sexually inexperienced, with completed and limited sexual experience). Afterwards, the males were exchanged in each group of females in the morning (08:00) and in the afternoon (18:00). This management was maintained for 15 days.

Behavioural measurements

For males, behavioural measure were performed with focal continued observation (Martin and Bateson 2007), on days 0, 1 and 2, for a period of 20 min (08:00 to 08:20 and 18:00 to 18:20). Individual total number of occurrences of ano-genital sniffing, nudging, self-urination, flehmen response, mounting attempts, mounts without and with intromission were recorded to verify that males were sexually active. During behavioural measure all males in contact with the three groups of females displayed sexual activity (Table 1; Figure 2 and Figure 3; Flores et al 2000; Loya-Carrera et al 2014).

Regarding to females we decided that measurements should be taken only during the first three days post-introduction of males into female groups, since it has been demonstrated to be the time lapse sufficient for photo-stimulated males induce sexual response in anestrous females through the male effect (Flores et al 2000; Rivas-Muñoz et al 2007; Bedos et al 2010). So that females were observed with a similar method as the described for the males, and the frequency of the next proceptive behaviours were recorded: tail wagging, female-female sniffing, female-male sniffing, female-female mounts, and emission of urine (Table 1). Receptive sexual behaviour registered was acceptance of the mount with intromission (Table 1). A female was considered as receptive when she remained immobile and accepted to be mounted by the male (Chemineau et al 1992). In addition, frequency of vocalizations (high bleating) was recorded. The observations were videotaped using a video camera (SONY-V8, Japan). The video-recordings were analyzed later in the laboratory using the program Observer Video Pro version 4.0 (Noduls, The Netherlands).

Table 1 Sexual behaviours in male goats in contact with females.

Behaviour	Description
Male	
Nudging	He approaches the female sometimes vocalizing and lowering his head
Ano-genital sniffing	He approaches his nose near the female (<0.005 m) smelling her genital or anal areas
Mounting attempts	He attempts to mount the female, but does not place its body on top
Flehmen response	After smelling and/or tasting the females' urine he lifts its head and upper the lip up
Self-urination	He urinates himself by turning his penis onto his face
Mounts without and with intromission	He mounts the female either penetrating or not penetrating her
Female	
Tail wagging	She moves its tail quickly sideways. The frequency is measured by the number of episode in a time unit
Female-female sniffing	When a female approaches another female in order to smell a part of her body
Female-female mounts	A female climbs up getting on top of another female
Emission of urine	She does urinate
Acceptance of mounts with intromission	A female stays still, allowing to be mounted and penetrated by a male

DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

Statistical analysis

The total frequency of behaviors as tail wagging, female-female sniffing, female-male sniffing, female-female mounts, acceptance of mounts, vocalizations, and emission of urine, were compared between groups of females using the Chi-square test for multiple group comparison and with the Fisher exact probability test. Statistical analysis was carried out using the statistical package SYSTAT version 13.00.05 (Systat Software, Inc., Chicago, IL, USA).

Results

Proceptive behaviours

Sexually inexperienced females and the group with complete sexual experience displayed higher frequency of tail wagging than those with limited sexual experience ($P<0.001$, Figure 4). Likewise, sexually inexperienced females displayed higher female-female sniffing ($P<0.001$; Figure 4) than those groups with complete and limited sexual experience. Furthermore, sexually inexperienced females displayed higher emission of urine ($P<0.05$; Figure 4) than those groups with complete and limited sexual experience. In contrast, the group with limited sexual experience displayed higher female-female mounts ($P<0.05$; Figure 4) than those groups inexperienced and with complete sexual experience. In addition, the group with complete sexual experience displayed higher female-male sniffing ($P<0.001$; Figure 4) than those groups inexperienced and with limited sexual experience.

Receptive behaviour

The frequency of acceptance of the mounts did not differ ($P>0.05$; Figure 4 and Figure 5) between the three groups of females.

Other behaviour

Sexually inexperienced females emitted higher vocalizations ($P<0.001$; Figure 4) than those groups with complete and limited sexual experience.

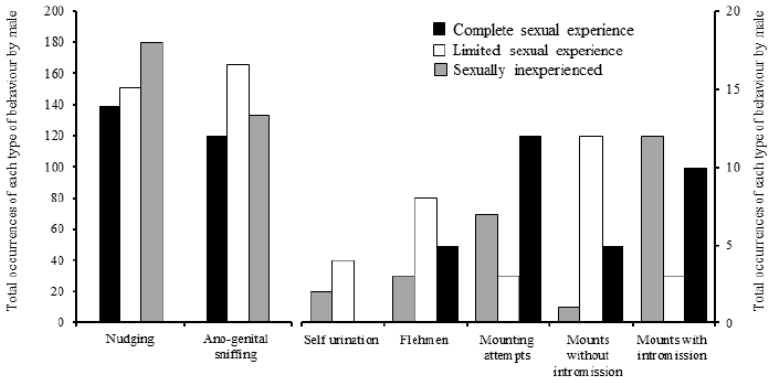

Figure 2 Individual occurrences of nudging, ano-genital sniffing, self-urination, flehmen response, mounting attempts, mounts without and with intromission in males interacting with sexually inexperienced females (gray bars), with complete sexual experienced females (dark bars) and with limited sexual experienced females (white bars). Sexual behaviour of males was observed from 08:00 to 08:20 and 18:00 to 18:20 on days 0, 1, and 2 after introduction of males in the groups of females. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15.

Discussion

The results of the present study indicate that sexually inexperienced anestrous goats were able to display proceptive and receptive sexual behaviours, as those sexually experienced goats, when they were exposed for the first time to photo-stimulated males. We considered in our study that in spite of the fact that sexually inexperienced females were exposed to a photo-stimulated male suddenly at 14 months of age, lack of sexual experience or familiarity to males did not prevent them to display sexual behaviours. In fact, sexual behaviour was similar between sexually inexperienced goats, and in those were already familiarized with the male completely or restricted since weaning. Our results also indicate that lack of sexual experience did not affect the response to the male effect during seasonal anestrous. This is relevant because it indicates that there are innate factors involved in the acceptance of mounts (receptivity) which are activated when females receive sensorial stimuli, in this case sexual bio-stimulation by photo-stimulated males (Pfaus et al

DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

2001; Delgadillo et al 2002). Then, when females exhibit proceptive behaviours learning factors are involved and facilitates mating (Agmo 1999; Pfaus et al 2001; Gelez et al 2004).

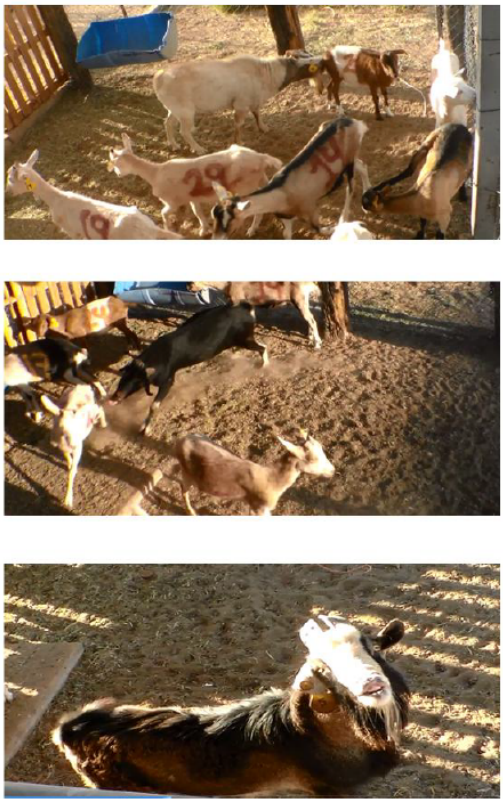

Figure 3 Sexual behaviours of a) ano-genital sniffing, b) nudging, and c) flehmen response exhibited by photo-stimulated males interacting with anestrous females. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15.

In the present study, sexual behaviour of tail wagging was more frequent in the groups with sexual inexperience and complete sexual experience than in the restricted females group. These results are in accordance with literature, because tail wagging also was observed in ewes, and did not differ between sexually inexperienced and experienced anestrous females (Hawken et al 2008). In addition, these behaviour also was observed in goats, during the proceptive phase, that were in induced estrus (Imwalle and Katz 2004; Haulenbeek and Katz 2011), and in females that were naturally in estrus, displaying not only tail wagging but also remaining close to the males (Llewelyn et al 1993). According to our results and those studies mentioned above, it is shown that tail wagging is a characteristic sign of estrus in both sheep and goats. Therefore, ewe and goat females display tail wagging as a visual signal to establish communication with the males.

The female-female sniffing was more observed in sexually inexperienced goats than in complete and limited sexual experience female groups. Perhaps this response was due to fact that sexually inexperienced goats were more familiarized with other females than males, and then they re-directed such behaviour, as has been demonstrated in male goats (Ungerfeld et al 2014).

The homosexual behaviours, i.e. when males exhibit sexual behaviours to individuals of the same sex, are more frequent in males that had been isolated from females (Ungerfeld et al 2014). In our study, anestrous females with limited sexual experience exhibited more female-female mounts than sexually inexperienced goats and those with complete sexual experience, which could be associated with an attempt to attract attention of the male, and initiate a sexual interaction, perhaps caused by previous restriction to males (Beach 1976; Shearer and Katz 2006). Llewelyn et al (1993) found that behaviour of female-female mounts was exhibited by goats with higher social rank which were in estrus during the natural breeding season also in order to attract attention of males.

In regards to female-male sniffing, females with complete sexual experience exhibited more often this behaviour than sexually inexperienced and those with limited sexual experience. This response was probably due to the fact that females already had sexual experience and identified easily the presence of males.

The receptive behaviour (acceptance of mounts) did not differ between the three groups of goats exposed to photo-stimulated males. In addition, this response was similar to that reported in sexually inexperienced goats (isolated from males) and in sexually experienced goats without sexual intromission before the male effect (Fernández et al 2011). Likewise, receptivity exhibited by sexually inexperienced females during their first exposure to photo-stimulated males was similar to exhibited by females with previous sexual experience, and exposed to photo-stimulated males (Rivas-Muñoz et al 2007; Luna-Orozco et al 2008; Loya-Carrera et al 2014).

In contrast, our results differed from those of Delgadillo et al (2012), where sexually inexperienced females and exposed for the first time to vocalizations of males, displayed lower acceptance of mounts than those experienced. These results suggest that total contact with males is necessary for females to exhibit sexual behaviours (Delgadillo et al 2012). In our study we found that sexually inexperienced females emitted more vocalizations and emissions of urine than other two groups of goats. This response was probably due to the fact that females suffered stress when they were placed in contact with the males for the first time, as was observed in ewes (Gelez et al 2004).

DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

Furthermore, the emissions of urine can also be considered as proceptivity behaviour as was demonstrated in mice (*Mus musculus*, Dizinno et al 1978). Also it has been shown that critical situations such as exposure to new stimuli (Chojnacki et al 2014), social isolation (Terrazas et al 2012) and hunger induce an increase in vocalizations, and eliminations (Poindron et al 2007). It has also been shown that goat females are more sensitive to respond that way than males (Terrazas et al 2012; Chojnacki et al 2014).

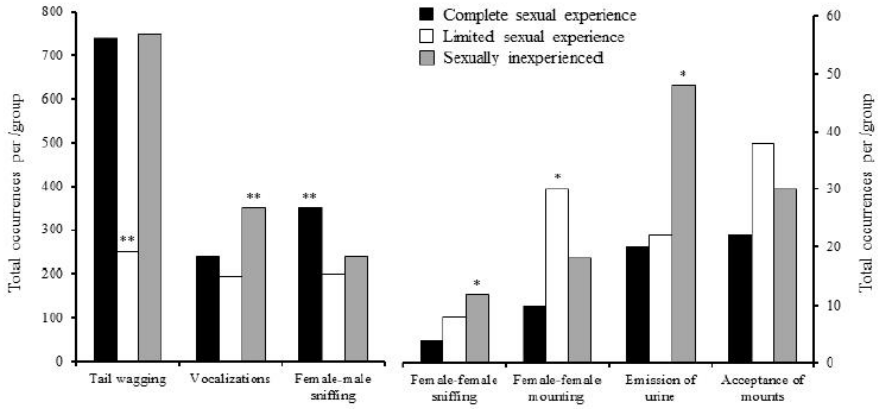

Figure 4 Frequencies of tail wagging, vocalizations, female-male sniffing, female-female sniffing, female-female mounts, emission of urine, and acceptance of mounts in sexually inexperienced females (gray bars), with complete sexual experienced females (dark bars), and with limited sexual experienced females (white bars). Sexual behaviour of females was observed from 08:00 to 08:20 and 18:00 to 18:20 on days 0, 1 and 2 after introduction of males in the groups of females. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15. *($P<0.05$), **($P<0.001$).

In the present study, our females deprived of the male presence before the male effect displayed sexual behaviours as those with previous experience. This response was due to the fact that females were exposed to males that exhibited high sexual behaviour. It is reported in literature that photo-stimulated males which exhibit a high level of sexual behaviour as nudging, ano-genital sniffing, self-urination, flehmen response, mounting attempts, and mounts with intromission are efficient for inducing sexual response in anestrous females. On the other hand, those males with low sexual behavior are inefficient to induce sexual response in anestrous females (Delgadillo et al 2002; Muñoz et al 2016). In addition, photo-stimulated males exhibiting sexual behaviour during natural sexual rest inducing a high percentage (>92%) of estrus (receptivity) in sexually experienced females (Rivas-Muñoz et al 2007; Luna-Orozco et al 2008). Finally, the results mentioned above suggest that photo-stimulated males first induce the proceptive phase, and then the receptive phase (acceptance of mounts) in anestrous females.

Figure 5 Receptivity sexual (acceptance of the mount) in sexually inexperienced females exposed to photo-stimulated males. Males were rendered sexually active by exposure to artificial long days (16 h of light by day) from November 1 to January 15.

DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

Conclusions

Sexually inexperienced females during anestrous season display proceptivity and receptivity behaviours as those sexually experienced, when exposed to photo-stimulated males for the first time through the male effect.

Acknowledgements

The authors are grateful to all members of the Centro de Investigación en Reproducción Caprina (CIRCA) of the Universidad Autónoma Agraria Antonio Narro for their support during the experiment. Jessica Anabel Loya-Carrera was supported by a scholarship of CONACyT during her doctoral studies.

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DOI http://dx.doi.org/10.14269/2318-1265/jabb.v5n2p64-71

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DOI http://dx.doi.org/10.14269/2318-1265/jabb.v3n2p64-71

ARTÍCULO 2

SEXUALLY INEXPERIENCED PHOTO-STIMULATED BUCKS ARE EFFICIENT TO STIMULATE SEXUAL ACTIVITY EITHER IN NULLIPAROUS OR MULTIPAROUS ANESTROUS GOATS

SUMMARY

In sheep, sexually inexperienced rams take longer to socialize when they have first contact with females, besides exhibit lower sexual behavior. In sheep and goats, nulliparous females exhibit lower sexual response than multiparous. The aim of this study was to determine whether sexually inexperienced photo-stimulated males display sexual behavior during the first contact with nulliparous females, and whether males could induce sexual activity in these females. Sexually inexperienced males (n = 6) were used. Males were subjected to a treatment of long days to induce sexual activity during the sexual rest. In April, during anestrous season, two groups of nulliparous and multiparous (n = 30/group), females were placed in contact with males (3/group of females) for 15 consecutive days. On the first day, the nudging did not differ between males in contact with nulliparous and multiparous females. While on days 1 and 2, the nudging were higher in males in contact with multiparous than nulliparous. On days 0 and 1, the ano-genital sniffing did not differ between males in contact with the two groups of females, while on day 2 were higher in nulliparous. In contrast, on days 0, 1, and 2, the mounting attempts, flehmen, self-urination, and mounts with and without intromission did not differ between males. Ovulations did not differ between nulliparous (96%) and multiparous (93%). Ovulation rate did not differ between nulliparous (1.6 ± 0.18) and multiparous (1.4 ± 0.75). Short (53% and 63%) and

normal cycles (43% and 30%) did not differ between the two groups of females. Pregnancy rate did not differ between nulliparous (83%) and multiparous goats (80%). It is concluded that sexually inexperienced photo-stimulated males are efficient to induce sexual activity either in nulliparous or multiparous anestrous goats.

INTRODUCTION

In small ruminants (sheep and goats) in the subtropics, there are some breeds that besides of their reproductive seasonality (Ortavant *et al*. 1988; Duarte *et al*. 2008), other factors also affect sexual response in both genders. In the case of the males, they are affected due to sexual experience (Katz *et al*. 1988), while females they are influenced by the number of parturitions (Murtagh *et al*. 1984). In sheep, sexually inexperienced rams (without any prior sexual contact with females), take longer time to initiate a sexual interaction, besides display lower frequency of sexual behaviors as mounting attempts and mounts with intromission (Katz *et al*. 1988; Price *et al*. 1991, 1994). In goats, sexually experienced photo-stimulated males, when exposed to females display high sexual behavior like the increase in the frequency of nudging, ano-genital sniffing, flehmen, mounting attempts, and mounts with intromission (Flores *et al*. 2000; Delgadillo *et al*. 2002; Loya-Carrera *et al*. 2014; Ponce *et al*. 2014). The efficiency of these photo-stimulated males has been evaluated when they are exposed to seasonally anestrous females through the male effect (Flores *et al*. 2000; Delgadillo *et al*. 2002; Muñoz *et al*. 2016).

In the case of the factor of parity some studies have demonstrated the impact of this fact. For example, in sheep, nulliparous ewes show lower ovulations and cyclicity than multiparous (Chanvallon *et al*. 2010). In fact, nulliparous ewes express estrus later when compared with multiparous (Martin *et al*. 2004). In goats, a study demonstrate that two to

three corpora lutea occur most often in multiparous than nulliparous females (Simões *et al*. 2008). Furthermore, nulliparous goats have shorter estrous cycles than multiparous (González-Stagnaro & Madrid-Bury 1993). The aforementioned studies also show that nulliparous ewes and goats have lower sexual response than multiparous. However, previous studies contrast with those results because in goats it was showed that sexual response (estrus and ovulation) was similar between nulliparous and multiparous goats (95% and 100%; 100% and 100%, respectively) when exposed to sexually experienced photo-stimulated males (Luna-Orozco *et al*. 2008). Therefore, the current study has as hypothesis that sexually inexperienced males submitted to a photo-periodic treatment of artificial long days, are able to display sexual behavior during the first contact with females, and sexual response induced did no differ between nulliparous and multiparous goats. The aim of this study was to determine whether sexually inexperienced photo-stimulated males display sexual behavior during the first contact with anestrous females, and evaluate if this response vary in the females according to their parity.

MATERIALS AND METHODS

Ethical note

The management of the animals in the present study was carried out following the protocol of the Norma Oficial Mexicana, according to specifications for the production, care and management of laboratory animals (SAGARPA 2001).

General conditions

Data were registered from February 2014 to May 2015. The experiment was carried out during the non-breeding season using local creole goats from the Laguna region in the State of Coahuila, Mexico (26°23'N, 104° 47'W, 1200 m a.s.l.). The Laguna region has a semi-arid climate and dry period last from November to May. In this region the photoperiod varies from 13.36 h of light at the summer solstice to 10.24 h of light at the winter solstice (Delgadillo 2011). While creole goats have it origin from populations brought from the Iberian peninsula, mainly from Andalucia, Extremadura and Canary Islands in the 16th century (de Alba 1987). Creole goats are derived from the Spanish Granadina, Murciana, and Malageña breeds. Afterwards, these goats were crossed with Alpine, Saanen, Toggenbourg and Anglo-Nubian breeds.

Males

Goat kids were born on 27 ± 2 days December 2013 (± SEM; mean value ± standard error of mean) during the sexual rest season. Males (n = 6) were weaned at 40-days-old, the body weight (BW) registered was 8.8 ± 0.22 kg. From this date until the next sexual rest season, males were kept in a pen of 5 x 8 m. In these conditions, males were kept in complete isolation from females. Males were fed with alfalfa hay *ad libitum* (18% crude protein, 1.95 Mcal/kg) and commercial concentrate (18% crude protein, 2.05 Mcal/kg) in order to cover their nutritional requirements until the end of the study. They also had free access to water and mineral salts during the study.

Photoperiodic treatment

When males were 11-months-old, their BW and BCS (body condition score) were 23.8 ± 0.91 kg and 2.5 ± 0.0, respectively; they were subjected to artificial long days (16 h

of light and 8 h of darkness), from 1 November to 15 January (Fig.1). The BCS scale used was the proposed by Walkden-Brown *et al.* (1997; 1, very thin and 4, obese). From this date, males perceived the natural photoperiod until the end of the experiment. In males born in this region, the natural sexual rest occurs from January to May (Delgadillo *et al.* 2002). The photoperiodic treatment induces sexual activity in March and April (increases odor, sperm production and sexual behavior; Delgadillo *et al.* 2002).

Females

Before introduction of the males, on 10 and 20 March, a transrectal ultrasonography was performed to determine the ovulatory state using ultrasound equipment Aloka SSD-500 (Tokyo, Japan) equipped with a 7.5 MHz transducer (Simões *et al.* 2007, Delgadillo *et al.* 2011). An anovulatory state was established in those females in whom a corpora lutea was not detected.

Before establishing the experiment, three days earlier, females were separated according to their parity. A nulliparous female was considered when it had not had experienced a parturition. While multiparous goats were those females who already have at least two parturitions. Females were divided into two groups: one was composed with nulliparous females (n = 30), their BW was 28.2 ± 0.4 kg and BCS was 1.9 ± 0.01, and the second group was composed with multiparous females (n = 30), their BW was 37.5 ± 0.7 kg and BCS was 2.1 ± 0.02. The average age of nulliparous and multiparous females was 1.2 and 3.4-years-old, respectively. Afterwards each group of nulliparous and multiparous was subdivided into 3 groups consisting of 10 females each.

During the study, multiparous females were milked daily in the morning. All females were fed with alfalfa hay *ad libitum* (18% crude protein, 1.95 Mcal/kg) and 200 g per day of commercial concentrate (14% crude protein, 2.5 Mcal/kg). Water and salt minerals were available at free access.

Male effect

Before male effect (three days earlier), males were 15-months-old and registered 39.5 ± 1.4 kg and 2.8 ± 0.1, of BW and BCS, respectively. On 30 March (08.00 h; day 0), males were placed in contact with nulliparous and multiparous females. The male–female ratio 1:10 was used. Males remained in contact with females for 15 consecutive days. Males were rotated into subgroups of females.

Measurement

Males

Males' sexual behavior was recorded with focal continuous observation during 1 h (08.00 to 09.00 h) on days 0, 1, and 2 post-introduction into pens containing females. Trained observers followed males individually and registered the frequency of nudging, ano-genital sniffing, flehmen, self-urination, mounting attempts, mounting with and without intromission (Flores *et al*. 2000; Loya-Carrera *et al*. 2014).

Females

Ovulations were inferred from plasma concentrations of progesterone. An ovulation was considered when the plasma progesterone level was ≥0.5 ng/ml. Short and normal cycles were determined. It was considered a short cycle when progesterone levels increased from day 2 to day 6 post-introduction of the male, then decreased to basal levels, and again

134 increased from 7 to 10 days. It was also considered a normal cycle when the level of
135 progesterone increased sustained from 2 to 7 days post-introduction of the male
136 (Chemineau *et al*. 2006; Bedos *et al*. 2010). The number of corpora lutea in each goat and
137 ovulation rate were determined on day 18 post-introduction of the males by transrectal
138 ultrasonography. Pregnancy rate (number of pregnant females/number of females exposed
139 to males) was obtained by abdominal ultrasonography at 50 days post-introduction of the
140 males. The same ultrasound equipment described earlier, but connected to the abdominal
141 probe of 3.5 MHz, was used for this purpose.

142 *Blood sampling and Immunoassay*

143 Blood samples to determine plasma progesterone level were carried out for 16
144 consecutive days (days 0–15) post-introduction of the males. Samples were collected by
145 venipuncture of the jugular vein, and they were placed in 5 ml tubes containing heparin (30
146 µl; Inhepar® PISA Laboratories, S.A. de C.V.) as an anticoagulant. Plasma was obtained
147 by centrifugation of the blood samples at 3500 × g for 30 min (centrifuge ORTO-ALRESA
148 Digitor 21 R, Madrid, Spain); then, the plasma was stored at -20 °C until hormone
149 concentrations were measured. The plasma progesterone concentration was determined by
150 radioimmunoassay (Cisbio Bioassays PROG-CTRIA) according to the technique described
151 by Grajales *et al*. (2010). The assay sensitivity was 0.05 ng/ml. The intra-assay coefficient
152 of variation was 9.13%.

153 *Statistical analysis*

154 In males, the total frequency of each sexual behavior was compared between groups
155 with the chi-square test. The proportions of females that ovulated, short and normal cycles,

and pregnancy rates were compared between groups with the Fisher's Exact test. Ovulation rate was compared between groups using the Mann–Whitney *U* test. Data are expressed as mean value ± standard error of mean, and differences were considered significant at the level of $P \leq 0.05$. Statistical analysis was performed by using the statistical package SYSTAT 13.00.05 (Evanston, IL, USA).

RESULTS

Sexual behavior of male goats

On day 0, the frequency of nudging did not differ between the males in contact with nulliparous and multiparous females ($P>0.05$; Fig.2). On days 1 and 2, the frequency of nudging was higher in males in contact with multiparous than nulliparous ($P<0.001$; Fig. 2). On days 0 and 1, the frequency of ano-genital sniffing did not differ between males in contact with nulliparous and multiparous females. However, on day 2, males in contact with nulliparous displayed higher ano-genital sniffing than multiparous ($P<0.001$; Fig. 2). On days 0, 1, and 2, mounting attempts, flehmen, self-urination, and mounts with and without intromission did not differ between males in contact with nulliparous and multiparous females ($P>0.05$, in all behaviours; Fig. 2).

Ovulatory and reproductive responses of goats exposed to males

Females that ovulated and their ovulatory rate did not differ between nulliparous and multiparous in contact with sexually inexperienced males ($P>0.05$ for each variable; Table 1). In addition, females that displayed short and normal cycles ($P>0.05$ each) did not differ

246 The aforementioned results could help us understand that with the prior application
247 of photoperiodic treatment, it is feasible to induce sexual behavior in sexually
248 inexperienced males during the sexual rest season, and stimulate sexual activity in
249 nulliparous or multiparous anestrous females.

250 CONCLUSION

251 It is concluded that sexually inexperienced photo-stimulated males exhibit sexual behavior
252 during the first contact with nulliparous anestrous goats, and they are able to stimulate
253 sexual activity in either nulliparous or multiparous anestrous females.

254

255 ACKNOWLEDGMENTS

256 This project received partial funding by Fundación Produce Coahuila, A.C. [grant number
257 CL151120]. The authors thank all members of the Centro de Investigación en
258 Reproducción Caprina (CIRCA) of the Universidad Autónoma Agraria Antonio Narro for
259 its support to carry out this study. They appreciate the support from José Alfredo Flores
260 Cabrera for carrying out the ultrasounds. Also thanks to Clara Murcia of the Departamento
261 de Reproducción Animal of the Facultad de Medicina Veterinaria y Zootecnia of the
262 Universidad Nacional Autónoma de México to carry out hormonal determinations. They
263 are grateful to Jesús Palomo Reyna for the care of the males, and to Andrés García
264 Martínez and Imelda Rodríguez Herrera for facilitating the goats of this study. J. Loya-
265 Carrera was supported by scholarship (441658) of CONACYT during her doctoral studies.

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394 **Figure legends**

395 Fig.1 A photoperiodic treatment of artificial long days (16 h of light and 8 h of darkness)
396 was applied to sexually inexperienced males from 1 November to 15 January. Afterwards
397 the males received the natural variations of day length. The pen had artificial shade and was
398 equipped with 6 lamps (68 W) that provided a light intensity of 300 lux at the lateral level
399 of the eyes of the males. The lamps were programmed to start in the morning at 06.00 h and
400 off at 08.00 h and in the evening from 18.00 to 22.00 h.

401 Fig. 2. Frequencies per day of nudging, ano-genital sniffing, flehmen, self-urination,
402 mounting attempts, mounts with and without intromission in sexually inexperienced photo-
403 stimulated males exposed to nulliparous (□) and multiparous (■) female goats. On days 1
404 and 2, males in contact with multiparous females displayed higher nudging, while on day 2,
405 males in contact with nulliparous females displayed higher ano-genital sniffing ($P<0.001$).
406 Sexual behaviors were observed 1 h (08.00 to 09.00 h) on days 0, 1, and 2 post-introduction
407 of the males into female groups. Males received a photoperiodic treatment by exposure to
408 artificial long days (16 h of light/day and 8 h of darkness) from 1 November to 15 January.

409 Fig. 3. Profiles of plasma progesterone concentrations in nulliparous (♦) and multiparous
410 (◊) female goats with short and normal cycles post-introduction of sexually inexperienced
411 photo-stimulated males. Males received an artificial photoperiodic treatment of long days
412 (16 h of light/day and 8 h of darkness) from 1 November to 15 January.

413

Fig. 1

Fig.1 A photoperiodic treatment of artificial long days (16 h of light and 8 h of darkness) was applied to sexually inexperienced males from 1 November to 15 January. Afterwards the males received the natural variations of day length. The pen had artificial shade and was equipped with 6 lamps (68 W) that provided a light intensity of 300 lux at the lateral level of the eyes of the males. The lamps were programmed to start in the morning at 06.00 h and off at 08.00 h and in the evening from 18.00 to 22.00 h.

190x254mm (96 x 96 DPI)

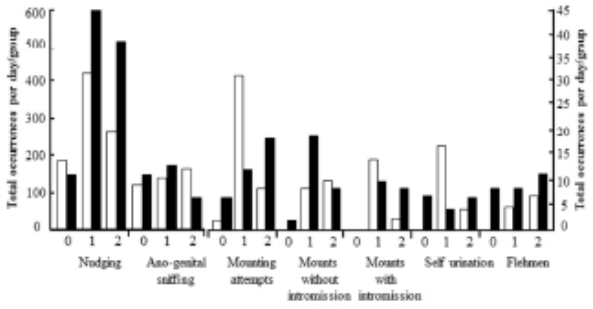

Fig. 2

Fig. 2. Frequencies per day of nudging, ano-genital sniffing, flehmen, self-urination, mounting attempts, mounts with and without intromission in sexually inexperienced photo-stimulated males exposed to nulliparous (□) and multiparous (■) female goats. On days 1 and 2, males in contact with multiparous females displayed higher nudging, while on day 2, males in contact with nulliparous females displayed higher ano-genital sniffing (P<0.001). Sexual behaviors were observed 1 h (08.00 to 09.00 h) on days 0, 1, and 2 post-introduction of the males into female groups. Males received a photoperiodic treatment by exposure to artificial long days (16 h of light/day and 8 h of darkness) from 1 November to 15 January.

225x300mm (96 x 96 DPI)

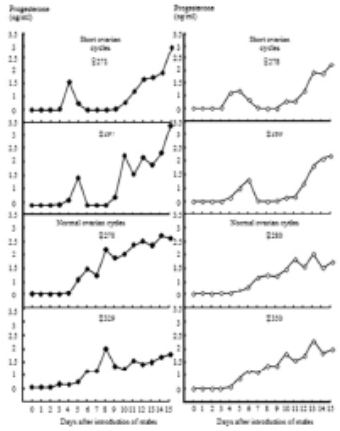

Fig. 3

Fig. 3. Profiles of plasma progesterone concentrations in nulliparous (♦) and multiparous (◇) female goats with short and normal cycles post-introduction of sexually inexperienced photo-stimulated males. Males received an artificial photoperiodic treatment of long days (16 h of light/day and 8 h of darkness) from 1 November to 15 January.

190x254mm (96 x 96 DPI)

Table 1. *Ovulatory and reproductive responses in nulliparous and multiparous anestrous goats in contact with sexually inexperienced photo-stimulated males. Males were rendered sexually active by exposure to artificial long days (16 h of light per day and 8 h of darkness) from 1 November to 15 January*

Groups	n	Females with ovulations (%)	Ovulation rate	± S.E.M.	Females with short ovarian cycles (%)	Females with normal ovarian cycles (%)	Pregnancy rate (%)
Nulliparous	30	96	1.6	0.18	53	43	83
Multiparous	30	93	1.4	0.75	63	30	80

± S.E.M. are mean value ± standard error of mean

CONCLUSIONES

Los resultados presentados en los dos artículos permiten concluir que:

1.-Los caprinos, tanto hembras, como machos sin experiencia sexual son capaces de mostrar conductas sexuales similares a aquellos caprinos con experiencia sexual previa.

2.- Además, los machos sin experiencia sexual foto-estimulados son capaces de inducir la respuesta sexual en las cabras anéstricas durante el efecto macho, no importando si son nulíparas o multíparas.

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