La reducción del tiempo de contacto diario entre machos y hembras no disminuye las respuestas ovulatoria y reproductiva de las cabras expuestas al efecto macho durante el anestro

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TESIS de co-tutela con la Université François Rabelais de Tours, Francia

PRESENTADA COMO REQUISITO PARCIAL
PARA OBTENER EL GRADO DE:

DOCTOR EN CIENCIAS AGRARIAS

UNIVERSIDAD AUTÓNOMA AGRARIA

ANTONIO NARRO

UNIDAD LAGUNA

Subdirección de Postgrado

Director de tesis: Dr. José Alberto Delgadillo Sánchez

Torreón, Coahuila, México

Marzo 2013

UNIVERSIDAD AUTÓNOMA AGRARIA

ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

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DOCTOR EN CIENCIAS AGRARIAS

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Torreón, Coahuila, México, Marzo 2013

CRÉDITOS

Esta tesis doctoral corresponde a los estudios realizados con una beca otorgada por el Gobierno de México, a través de la Secretaría de Relaciones Exteriores. El presente trabajo se realizó en el Centro de Investigación en Reproducción Caprina de la Universidad Autónoma Agraria Antonio Narro, Unidad Laguna, bajo la dirección del Dr. José Alberto Delgadillo Sánchez.

AGRADECIMIENTOS

A la Universidad Autónoma Agraria Antonio Narro, por las facilidades brindadas durante mis estudios de Doctorado.

Al Programa de Cooperación de Postgrado (PCP) del Consejo Nacional de Ciencia y Tecnología (CONACyT), por el apoyo económico otorgado para mi movilidad y la de los investigadores que participaron en la realización de este proyecto.

A mi asesor principal, Dr. José Alberto Delgadillo Sánchez, quién me recibió para una estancia en su centro de investigación hace 5 años y me transmitió su pasión por la reproducción caprina. Por su apoyo, confianza y comprensión (gracias a su gran conocimiento de la cultura francesa!) en esta etapa de mi vida, y sobre todo por sus enseñanzas. Muchas gracias!

A mi comité particular de asesoría: Dr. Matthieu Keller, Dr. José Alfredo Flores Cabrera, Dr. Raúl Paredes Guerrero, Dr. Philippe Chemineau y Dra. Hélène Gelez, por su tiempo, consejos y por todo su apoyo para la realización de esta tesis.

A la Dra. Ilda Graciela Fernández, quien fue mi anfitriona durante mi primera estancia en el CIRCA, y por su ayuda durante mis estudios de Doctorado.

Al Dr. Gerardo Duarte Moreno, por su valioso apoyo durante los trabajos experimentales y por compartirme su experiencia.

Al Dr. Horacio Hernández Hernández, por su ayuda en los análisis estadísticos de estos trabajos.

A los Drs. Jesús Vielma Sifuentes y Gonzalo Fitz Rodríguez por brindarme su ayuda y consejos para la realización de esta tesis.

Al Dr. Pascal Poindron, por sus valiosos consejos durante la redacción de los artículos científicos presentados en esta tesis.

Al Dr. Benoît Malpaux, quién me relacionó con el Dr. José Alberto Delgadillo y dedicó tiempo a la discusión de los protocolos de la presente tesis.

A Anne-Lyse Lainé, Corinne Laclie, Magali Saint Martin y todos los miembros del laboratorio de determinaciones hormonales del INRA de Tours, Francia, por la realización de los ensayos hormonales presentados en esta tesis.

A Esther Peña Revuelta y Dolores López Magaña por su apoyo durante mi estancia en el Postgrado y en el CIRCA, respectivamente.

A mis compañeros y amigos de Postgrado: M.C. Hillary Velázquez Ortega, M.C. Juan Carlos Martínez Alfaro, M.V.Z. Sergio Secundino Méndez y M.C.

Alfonso Muñoz Benítez, por su apoyo tanto técnico como moral durante mis estudios, por nuestros intercambios y su amistad.

Al caprinocultor Alejandro Sandoval y su familia, por haberme facilitado las hembras caprinas para la realización de mis 3 estudios de doctorado.

A mis amigos: Julie Debru, Caroline Léonardi et alexandre Alline: J'vous kiffe j'vous flashe, j'vous boule, j'vous love! Tatiana Chilovsky, David Belliard, Goulven Maréchal, Mathieu Laissus, Alexandre Treiber, Caroline Bachèlery, Thomas Desvignes, Thomas Sarrazin, Benoît Piquemal et Arnaud Deladerière: ENSAR forever!! Et pour tous: Bien qu'éparpillés aux 4 coins du monde, vous avez été d'un grand soutien dans cette étape de ma vie (merci Facebook, Skype et autre!).

Y a todas las personas que de alguna forma me acompañaron y ayudaron en este proyecto.

A TODOS USTEDES MUCHAS, MUCHAS, MUCHAS GRACIAS

DEDICATORIA

Quiero dedicar esta tesis a mis padres, Michel y Aline, quienes me han brindado su preciado apoyo en todos los grandes proyectos de mi vida, aunque fuera a miles de kilómetros de ellos… ¡Los amo!

A mi hermana Manou, por ser una persona tan linda y un ejemplo para mí. Espero tener un día una familia tan bonita como la tuya. ¡Te amo!

En especial a ti Beto, por tus consejos (sin ti no sería la casi mexicana que soy), por tu paciencia y por amarme. Eres la razón de mi vida y me motivas a ser una mejor persona cada día. ¡TE AMO!

"Vive como si fueras a morir mañana. Aprende como si fueras a vivir para siempre"

Mohandas Karamchand Gandhi

COMPENDIO

La reducción del tiempo de contacto diario entre machos y hembras no disminuye las respuestas ovulatoria y reproductiva de las cabras expuestas al efecto macho durante el anestro

Por

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Torreón, Coahuila, México, Marzo de 2013

La introducción de un macho cabrío o un carnero en un grupo de hembras anovulatorias estimula el comportamiento de estro y la ovulación en los primeros 5 días de contacto con los machos. Esta técnica de bioestimulación sexual se conoce como efecto macho. Las respuestas ovulatoria y reproductiva de las hembras expuestas al efecto macho puede variar con la intensidad del comportamiento sexual de los machos. El uso de machos cabríos inducidos a un comportamiento sexual intenso durante el

periodo de reposo sexual al someterlos a un tratamiento de días largos -machos foto-estimulados- incrementa considerablemente los porcentajes de cabras que ovulan, se gestan y paren al someterlas al efecto macho (Flores et al., 2000; Delgadillo et al., 2002). La duración del tiempo de contacto entre ambos sexos, es otro factor que influye sobre la respuesta ovulatoria de las hembras expuestas al efecto macho. En ovejas, es necesario mantener el contacto entre machos y hembras 24 h por día durante 13 días para que ovule la mayoría de las hembras (61 %; Signoret *et al*., 1982). En cambio, Rivas-Muñoz *et al*. (2007) demostraron que en las cabras locales de la Comarca Lagunera, la duración de contacto con machos cabríos foto-estimulados puede ser reducida a 16 h por día sin que se disminuya la respuesta estral de las hembras. Sin embargo, no se sabe si el tiempo de contacto entre machos cabríos foto-stimulados y cabras anéstricas se puede reducir aun más, sin disminuir la respuesta ovulatoria y reproductiva de las hembras. Por lo tanto, se realizaron 2 estudios para determinar si una reducción del tiempo de contacto diario entre los sexos no disminuye la respuesta ovulatoria y reproductiva de las cabras anéstricas expuestas al efecto macho. Dado que la reducción del tiempo de contacto no disminuyó la respuesta reproductiva de las hembras, en un tercer estudio se determinó si los machos foto-estimulados eran capaces de estimular 3 grupos de hembras al estar en contacto con ellas 4 h por día durante 15 días.

Estudio 1. En este estudio se realizaron 2 experimentos en años consecutivos para determinar las respuestas ovulatoria y reproductiva de cabras anovulatorias expuestas menos de 16 h diarias a machos cabríos foto-estimulados. En ambos experimentos se utilizaron hembras y machos cabríos locales de la Comarca Lagunera, ubicada en el subtrópico mexicano (26ºN). En el experimento 1 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, seguido del fotoperiodo natural, para estimular su actividad sexual durante el periodo de reposo (n=8). Las cabras multíparas y anovulatorias que se utilizaron se dividieron en 5 grupos: el grupo control fue aislado de los machos (n=9); los otros 4 grupos de hembras se expusieron a los machos foto-estimulados (n=2 machos/grupo) durante 16, 12, 8 o 4 h por día, respectivamente, por 15 días consecutivos a partir del 12 de marzo (día 0; n=17, 15, 15 y 15, respectivamente). Después del contacto con los machos, éstos eran retirados y alojados en corrales de “descanso” sin presencia de hembras. Las cabras expuestas al afecto macho permanecieron todo el tiempo en los corrales donde se ponían en contacto con los machos. En el experimento 2, los machos se sometieron al tratamiento fotoperiódico descrito en el experimento 1 (n=8). En este experimento, las hembras multíparas y anovulatorias se dividieron en 5 grupos como en el experimento 1: un grupo control (n=12) que fue aislado de los machos y otros 4 grupos (n=18 cada uno) que se expusieron a los machos foto-estimulados (n=2 machos/grupo) por duraciones de contacto iguales a las descritas en el experimento 1 a partir del 27 de marzo (día 0).

Después del contacto con los machos, las hembras se llevaban a corrales de “descanso” en los que no había existido la presencia de machos, es decir, libres del olor de éstos. En los experimentos 1 y 2, las ovulaciones y tasas ovulatorias se determinaron por la presencia y número de cuerpos lúteos a través de ultrasonografía transrectal a los 6 y 20 días después del primer contacto entre hembras y machos. Además, en el experimento 1, la ovulación se confirmó a través de los niveles plasmáticos de progesterona en las muestras sanguíneas que se obtuvieron del día 3 al 9 y el día 19 post introducción de los machos. En el experimento 1 y 2, el número de hembras gestantes en cada grupo se determinó a los 52 días post introducción de los machos mediante ultrasonografía abdominal. Más del 90 % de las hembras expuestas a los machos ovularon, mientras que la ovulación se registró solamente en el 11 y 0 % de las hembras de los grupos control en los experimentos 1 y 2, respectivamente ($P<0.001$). Además, los porcentajes de hembras que ovularon no fueron diferentes en los grupos en contacto con los machos ($P=1$). En los experimentos 1 y 2, las tasas ovulatorias y de preñez no difirieron entre los grupos expuestos a los machos foto-estimulados ($P\geq0.41$ y $P\geq0.50$, respectivamente). En conclusión, 4 h de contacto diario con machos foto-estimulados es suficiente para estimular las actividades ovulatoria y reproductiva de cabras anéstricas. Además, esta respuesta no es una consecuencia de la impregnación de las instalaciones por las señales olfativas del macho, pues la ovulación se produjo también en las cabras que permanecieron en corrales de “descanso” donde no había existido presencia de machos.

Estudio 2. El objetivo de este estudio fue determinar las respuestas ovulatoria y reproductiva de cabras anovulatorias expuestas menos de 4 h diarias a machos cabríos foto-estimulados. Se utilizaron hembras y machos cabríos locales de la Comarca Lagunera, ubicada en el subtrópico mexicano (26ºN). Los machos se sometieron al tratamiento fotoperiódico descrito en el estudio 1 (n=6). Las cabras multíparas y anovulatorias se dividieron en 4 grupos: el grupo control que fue aislado de los machos (n=20); otros 3 grupos de hembras se expusieron a los machos foto-estimulados (n= 2 machos/grupo) durante 4, 2 o 1 h por día por 15 días consecutivos a partir del 25 de marzo (día 0; n=18, 22 y 21, respectivamente). La ovulación fue determinada a través de los niveles plasmáticos de progesterona en las muestras sanguíneas que se obtuvieron del día 1 al 10 y el día 18 post introducción de los machos. Las tasas ovulatorias se determinaron por el número de cuerpos lúteos observados por ultrasonografía transrectal 18 días post introducción de los machos. El número de hembras gestantes en cada grupo se determinó a los 56 días post introducción de los machos mediante ultrasonografia abdominal. La fertilidad y la prolificidad se determinaron al parto. Más del 89 % de las hembras expuestas a los machos ovularon, mientras que solo el 5 % de las cabras del grupo control ovuló ($P<0.001$). Los porcentajes de hembras que ovularon no fueron diferentes en los grupos en contacto con los machos ($P=0.28$). En los grupos expuestos a machos, las tasas ovulatorias no difirieron según la duración diaria de contacto con

ellos (P=0.13). Además, las tasas de preñez, la fertilidad y la prolificidad no fueron diferentes según la duración diaria de contacto con los machos (P=0.15, P=0.20 y P=0.86, respectivamente). En conclusión, 1 h de contacto diario con machos foto-estimulados es suficiente para estimular las actividades ovulatoria y reproductiva de cabras anéstricas.

Estudio 3. El objetivo de este estudio fue determinar si los machos cabríos foto-estimulados eran capaces de estimular las actividades ovulatoria y reproductiva de 3 grupos diferentes de hembras anovulatorias al estar en contacto con ellas 4 h por día. Se utilizaron machos y hembras locales de la Comarca Lagunera ubicada en el subtrópico mexicano (26ºN). Los machos se sometieron al tratamiento fotoperiódico descrito en el estudio 1. El grupo control de machos (n=3) estuvo en contacto solamente 4 h por día con el grupo control de hembras (n=25), de 8:00 h a 12:00 h. Los machos experimentales (n=3) estuvieron en contacto con tres grupos experimentales de hembras de manera consecutiva: con el primer grupo (n=27) de 8:00 h a 12:00 h; con el segundo grupo (n=26) de 12:00 h a 16:00 h y con el tercer grupo (n=27) de 16:00 h a 20:00 h. Las hembras fueron expuestas a los machos por 15 días a partir del 25 de marzo (día 0). Después de terminarse el tiempo de contacto con las hembras, los machos permanecían en otro corral distante de las hembras hasta el día siguiente. La ovulación y las tasas ovulatorias fueron determinadas por la presencia y el número de cuerpos lúteos, respectivamente, a través de ultrasonografía

transrectal realizada a los 20 días post introducción de los machos. El número de hembras gestantes en cada grupo se determinó a los 60 días post introducción de los machos mediante ultrasonografia abdominal. La fertilidad y la prolificidad se determinaron al parto. En el grupo control como en los tres grupos experimentales, más del 85 % de las hembras ovularon y no hubo diferencias entre estos grupos (P≥0.67) o entre los tres grupos experimentales (P≥0.67). Además, las tasas ovulatorias no difirieron significativamente entre el grupo control y los grupos experimentales o entre los tres grupos experimentales (P=0.62 y P=0.42, respectivamente). Los machos fueron capaces de fertilizar a más del 72 % de las hembras, independientemente del número de grupos con los que tuvieron contacto (P≥0.17). Finalmente, más del 58 % de las hembras parieron y la fertilidad fue similar entre el grupo control y los grupos experimentales (P=1), así como entre los tres grupos experimentales (P≥0.77). Estos resultados permiten concluir que los machos cabríos foto-estimulados son capaces de inducir las actividades ovulatoria y reproductiva en 3 grupos sucesivos de hembras anovulatorias aun cuando la duración del contacto entre los sexos es reducida a 4 h por día.

Palabras claves: Efecto macho, Hembras anovulatorias, Duración de contacto, Ovulación, Olfato, Proporción macho-hembra.

ABSTRACT

The introduction of a buck or ram in a group of anovulatory females stimulates estrus behavior and ovulation within the first 5 days of contact with males. This technique of biostimulation is known as the male effect. Ovulatory and reproductive responses of females exposed to the male effect may vary with the intensity of male sexual behavior. The use of bucks induced to an intense sexual behavior during the non-breeding season by subjecting them to a long-day treatment -photo-stimulated males- increases significantly the percentages of goats ovulating, getting pregnant and giving birth in response to the male effect (Flores *et al*., 2000; Delgadillo *et al*., 2002). The duration of contact between males and females is another factor that modulates the response of females exposed to the male effect. In sheep, it is necessary to maintain contact between males and females 24 h per day for 13 days so that most females ovulate (61%; Signoret *et al*., 1982). On the contrary, Rivas-Muñoz *et al*. (2007) demonstrated that in local goats from the "Comarca Lagunera", reducing contact with photo-stimulated bucks from 24 h to 16 h per day did not reduce the response of females submitted to the male effect. However, it remains unknown if the duration of contact between photo-stimuladed bucks and anestrous goats can be further reduced without decreasing the ovulatory and reproductive responses of females. Therefore, two studies were carried out to determine whether a decrease of the duration of contact between sexes does not reduce the ovulatory and reproductive

responses of anestrous goats exposed to the male effect. Since the decrease of the duration of contact to 4 daily hours did not reduce the sexual and reproductive response of females, in a third study, we determined whether the photo-stimulated bucks were able to stimulate 3 groups of females being in contact with them 4 daily hours.

Study 1. Two experiments were carried out in two consecutive years during the non-breeding season to determine the ovulatory and reproductive responses of anestrous goats exposed less than 16 h per day to photo-stimulated bucks. In both experiments, local goats and bucks from the "Comarca Lagunera", located in subtropical Mexico (26ºN), were used. In experiment 1, adult males were subjected to a 2.5-month treatment of long days (16 h of light) from November 1st, followed by natural photoperiod, to stimulate their sexual activity during the non-breeding season (n=8). Multiparous anestrous goats were divided into 5 groups: the control group was isolated from males (n=9); the other 4 groups of females were exposed to the photo-stimulated males (n= 2 males/group) for 16, 12, 8 or 4 h per day, respectively, during 15 consecutive days from March 12th (day 0; n=17, 15, 15 y 15, respectively). After the period of contact, males were placed until next day in separated "resting" pens without females. Females in contact with males remained in the same pens where they were put in contact with them throughout the experiment. In experiment 2, males were subjected to the same photoperiodic treatment described in experiment 1 (n=8). In this

experiment, multiparous anestrous goats were divided into 5 groups as in experiment 1: the control group was isolated from males (n=12); the other 4 groups of females (n= 18 each) were exposed to the photo-stimulated males (n=2 males/group) for the same durations described in experiment 1, during 15 consecutive days from March 27th (day 0). After the period of contact, females were placed until next day in separated “resting” pens that had not contained bucks, that is, free of males’ odor. In experiment 1 and 2, ovulations and ovulation rates were assessed by the presence and number of corpora lutea observed in each female by transrectal ultrasonography 6 and 20 days after introduction of the bucks. Moreover, in experiment 1, ovulation was confirmed by progesterone levels determined in blood samples obtained from day 3 to 9 y on day 19 after introduction of the bucks. In experiments 1 and 2, the number of pregnant goats in each group was determined by abdominal ultrasonography 52 days after exposure to males. More than 90 % of females exposed to males ovulated, whereas only 11 or 0 % of the control females of experiment 1 and 2 did so, respectively ($P<0.001$). Moreover, the proportions of females that ovulated did not differ among groups of goats exposed to the photo-stimulated males ($P=1$). In experiments 1 and 2, ovulation and pregnancy rates were not different among groups of goats exposed to the photo-stimulated males ($P\geq0.41$ and $P\geq0.50$, respectively). To conclude, 4 h of daily contact with photo-stimulated males is sufficient to stimulate ovulatory and reproductive activities in seasonal anestrous goats. Moreover, this response is not a consequence of the impregnation of the pens with olfactory cues from the males, as ovulation

also occurred in females that were moved to the “resting” pens that had not contained bucks.

Study 2. The objective of this study was to determine the ovulatory and reproductive responses of anestrous goats exposed less than 4 h per day to photo-stimulated bucks. Local goats and bucks from the “Comarca Lagunera”, located in subtropical Mexico (26ºN) were used. Bucks were subjected to a photoperiodic treatment as described in study 1 (n=6). Multiparous anestrous goats were divided into 4 groups: the control group was isolated from males (n=20); the other 3 groups of females were exposed to the photo-stimulated males (n= 2 males/group) for 4, 2 or 1 h per day, respectively, during 15 consecutive days from March 25th (day 0; n=18, 22 y 21 respectively). Daily blood samples were taken from day 1 to 10 and 18 after exposure to males to assess ovarian activity by measuring the plasma concentrations of progesterone. In addition, ovulation rate was assessed by the number of corpora lutea observed in each female by transrectal ultrasonography 18 days after introduction of the bucks. The number of pregnant goats in each group was determined by abdominal ultrasonography 56 days after exposure to males. Fertility and prolificacy were determined at parturition. More tan 89 % of females exposed to the males ovulated whereas only 5 % of the control group females did so ($P<0.001$). The proportions of females that ovulated did not differ among groups of goats exposed to the photo-stimulated males ($P=0.28$). In the groups exposed to

males, ovulation rates did not differ depending on the daily duration of contact with males (P=0.13). Moreover, pregnancy rates, fertility and prolificacy did not differ depending on the daily duration of contact with males (P=0.15, P=0.20 and P=0.28, respectively). To conclude, one daily hour of contact with photo-stimulated males is sufficient to stimulate ovulatory and reproductive activities in seasonal anestrous goats.

Study 3. The objective of this study was to determine whether photo-stimulated bucks were able to induce the ovulatory and reproductive activities of three different groups of anestrous goats when interacting with them 4 h per day during 15 consecutive days. Local goats and bucks from the "Comarca Lagunera", located in subtropical Mexico (26ºN) were used. Bucks were subjected to a photoperiodic treatment as described in study 1. Control males (n=3) were in contact with the control group of females (n=25) from 8:00 h to 12:00 h. Experimental males (n=3) were in contact with three successive groups of females: with the first group (n=27) from 8:00 h to 12:00 h; with the second group (n=26) from 12:00 h to 16:00 h and with the third group (n=27) from 16:00 h to 20:00 h. Females were exposed to bucks for 15 days from March 25th (day 0). After the daily period of contact with females, bucks were placed together until the next day in a distant pen. Ovulations and ovulation rates were assessed by the presence and number of corpora lutea, respectively, observed in each female by transrectal ultrasonography 20 days after introduction of the bucks. The number of

pregnant goats in each group was determined by abdominal ultrasonography 60 days after exposure to males. Fertility and prolificacy were determined at parturition. In the control group as in the experimental ones, more than 85 % of females ovulated and these proportions did not differ between control females and experimental ones (P≥0.67) or between the three groups of experimental females (P≥0.67). Moreover, ovulation rates did not differ between control females and experimental ones or between the three groups of experimental females (P=0.62 and P=0.42, respectively). Males were able to fertilize more than 72 % of females, independently of the number of females they were exposed to (P>0.17). Finally, more than 58 % of females kidded and fertility did not differ between control females and experimental ones (P=1) or between the three groups of experimental females (P≥0.77). Those results enable to conclude that photo-stimulated bucks are able to induce the ovulatory and reproductive activities in three successive groups of anestrous goats even when the period of contact between sexes is reduced to 4 h per day.

Key words: Male effect, Anovulatory does, Duration of contact, Ovulation, Olfaction, Male–female ratio.

RÉSUMÉ

Certaines races caprines et ovines originaires ou adaptées aux régions subtropicales sont saisonnières. L'existence d'une alternance entre saison reproductrice et repos sexuel ou anoestrus chez le mâle et la femelle, respectivement, limite la reproduction à une période de l'année et surtout la rend impossible à contre-saison. Chez la femelle, la saisonnalité de la reproduction peut être modifiée à l'aide des relations socio sexuelles. Ainsi, la réintroduction d'un mâle dans un groupe de femelles en anoestrus saisonnier peut stimuler leur activité ovarienne dès les 10 premiers jours de contact. Cependant, la réponse des femelles à l'effet mâle peut varier en fonction de différents facteurs. Par exemple, l'intensité du comportement sexuel des mâles influence fortement la réponse des femelles en contact avec des mâles. L'utilisation de boucs induits à un comportement sexuel intense pendant la période de repos sexuel au moyen d'un traitement lumineux –mâles photo-stimulés- permet d'assurer la qualité de la stimulation fournie par les mâles. Le temps de contact entre mâles et femelles est également un facteur pouvant moduler la réponse des femelles et il a longtemps été suggéré qu'il devait être maintenu de manière continue (24h/24) pendant plusieurs jours afin que la majorité des femelles ovulent.

L'objectif de cette thèse a été de déterminer si une réduction du temps de contact journalier entre mâles et femelles diminue les réponses ovulatoire et reproductrice de chèvres en anoestrus saisonnier exposées aux mâles.

Dans une première étude, nous avons observé que 4 h de contact par jour avec des mâles photo-stimulés sont suffisantes pour stimuler les activités ovulatoire et reproductrice de chèvres en anoestrus saisonnier. De plus, cette réponse ne peut pas être attribuée à l'imprégnation des enclos par l'odeur des mâles car l'ovulation a aussi eu lieu chez les femelles qui étaient déplacées en dehors des périodes de contact dans des enclos où il n'y avait jamais eu la présence de mâles. Dans une seconde étude, nous avons mis en évidence que le temps de contact peut être encore réduit jusqu'à 1 h sans que les réponses ovulatoire et reproductrice ne soient diminuées. Cependant, il semble que la réponse ovulatoire soit retardée chez les femelles en contact avec les mâles 1 h par jour. En effet, la proportion d'entre elles ovulant dans les 4 jours après l'introduction du mâle est inférieure à celle des femelles en contact 4 h par jour. Néanmoins, après 15 jours de contact entre mâles et femelles, cette différence est compensée et les proportions de femelles ovulant sont élevées et similaires entre les groupes de femelles en contact avec les mâles, indépendamment du temps de contact. Enfin, dans une troisième étude, nous avons montré que les mâles photo-stimulés sont capables d'induire les activités ovulatoire et reproductrice de 3 groupes successifs de femelles lorsque la durée de contact est réduite à 4h par jour.

Les résultats de ce travail ouvrent de nouvelles perspectives quant à la conduite de la reproduction caprine en élevage. En effet, ils mettent en évidence la possibilité d'utiliser l'effet mâle de façon beaucoup plus efficace.

De plus, les résultats appellent à approfondir la connaissance des mécanismes neuroendocriniens à l'origine de la réponse des femelles observée dans ce travail. Par exemple, il serait intéressant de déterminer les profils de sécrétion de LH de femelles en contact continu ou intermittent avec des mâles.

Mots-clés : Saisonnalité, Effet mâle, Anoestrus, Durée de contact, Ovulation, Proportion mâle :femelles.

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INTRODUCCIÓN

En los caprinos y ovinos originarios de latitudes templadas, así como en algunas razas originarias o adaptadas a latitudes subtropicales, el fotoperiodo es la señal ambiental principal responsable de la estacionalidad sexual. Las variaciones del fotoperiodo modulan la retroalimentación negativa de la testosterona y del estradiol sobre el eje gonadotrópico, y es el mecanismo neuroendocrino responsable de la alternancia entre periodos de actividad y reposo sexual en caprinos y ovinos (Pelletier y Ortavant, 1975; Karsch *et al.*, 1980; Mori *et al*., 1987; Chemineau *et al*., 1988). En machos y hembras de estas dos especies, la sensibilidad a la retroalimentación negativa de la testosterona y del estradiol, respectivamente, aumenta durante los días largos, lo que disminuye la secreción de GnRH y LH, provocando los periodos de reposo sexual estacional (Pelletier y Ortavant, 1975; Karsch *et al*., 1980). La estacionalidad reproductiva tiene consecuencias sobre la producción de leche y carne. En efecto, en cabras y ovejas de zonas templadas, por ejemplo, los partos se concentran en unos meses del año, lo que provoca que la producción de leche y carne sea mayor durante el invierno y la primavera y menor durante el resto del año (Chemineau *et al*., 2007). Para evitar la estacionalidad productiva, es necesario tener un buen conocimiento de las estrategias reproductivas de las razas caprinas y ovinas para manejar su actividad reproductiva de manera pertinente durante el anestro estacional.

En los machos cabríos, los tratamientos fotoperiódicos basados en la sucesión de un periodo de días largos artificiales seguidos del fotoperiodo natural permiten inducir la actividad sexual de los machos fuera de la estación reproductiva natural. En los machos cabríos de la Comarca Lagunera en el norte de México (26ºN), la aplicación de 2.5 meses de días largos a partir del 1 de noviembre seguidos del fotoperiodo natural, estimula la secreción de testosterona, el comportamiento sexual y el olor de febrero a abril, durante el periodo de reposo sexual natural (Delgadillo *et al*., 2002; Rivas-Muñoz *et al*., 2007; Delgadillo y Vélez, 2010). Estos tratamientos fotoperiódicos se pueden aplicar en hembras para estimular su actividad sexual en el anestro estacional. Sin embargo, el uso de las relaciones socio-sexuales, y en particular del efecto macho, permite inducir la actividad sexual en cabras en el anestro estacional. La introducción de un macho en un grupo de hembras anovulatorias estimula la secreción de LH, el comportamiento de estro y la ovulación en los primeros 5 días de contacto con los machos (Flores *et al*., 2000; Delgadillo *et al*., 2002; Vielma *et al*., 2008; Fernández *et al*., 2011). Esta técnica de control reproductivo tiene la ventaja de no utilizar hormonas exógenas y evitar dispersar residuos potencialmente tóxicos en el medio ambiente. Sin embargo, la respuesta de las hembras expuestas al efecto macho puede variar en función de algunos factores como la intensidad del comportamiento sexual de los machos y la duración del tiempo de contacto entre ambos sexos (Signoret *et al*., 1982; Lindsay *et al*., 1992; Perkins y Fitzgerald, 1994; Delgadillo *et al*., 2006). En ovejas se observó que es necesario mantener el contacto entre machos y hembras 24 h por día durante 13 días para que ovule la mayoría de las

hembras (Signoret *et al*., 1982). En efecto, solo el 18 % de las hembras ovularon cuando se expusieron a los machos por 24 h, mientras que el 53 y el 61 % de ellas ovularon cuando estuvieron en contacto con los machos por 4 o 13 días, respectivamente (Signoret *et al*., 1982). Asimismo, en las cabras de la raza Cashemere, solamente el 24 % de las hembras ovularon cuando se expusieron 16 h por día a los machos durante 10 días (Walkden-Brown *et al*., 1993). Sin embargo, Rivas-Muñoz *et al*. (2007) demostraron que en las cabras locales de la Comarca Lagunera, la duración de contacto con machos cabríos foto-estimulados puede ser reducida a 16 h por día sin que se disminuya la respuesta estral de las hembras. Estos resultados obtenidos con cabras del subtrópico mexicano sugieren que el contacto de 24 h por día con machos cabríos foto-estimulados, no es necesario para obtener un efecto macho exitoso. Sin embargo, no se sabe si el tiempo de contacto entre machos cabríos foto-stimulados y cabras anéstricas se puede reducir aun más, sin disminuir la respuesta ovulatoria y reproductiva de las hembras.

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

1. Determinar si los machos foto-estimulados son capaces de estimular la actividad sexual de cabras anovulatorias al estar en contacto con ellas menos de 16 h diarias durante 15 días consecutivos.
2. Determinar si los machos foto-estimulados son capaces de estimular la actividad sexual de cabras anovulatorias al estar en contacto con ellas menos de 4 h diarias durante 15 días consecutivos.

3. Determinar si los machos foto-estimulados son capaces de estimular la actividad sexual de 3 grupos diferentes de hembras anovulatorias al estar en contacto con ellas 4 h diarias durante 15 días consecutivos.

REVISIÓN DE LITERATURA

1 Estacionalidad sexual en caprinos y ovinos

La selección natural ha dotado a los mamíferos de estrategias predictivas que aseguran que la actividad reproductiva ocurra en algunos meses del año para que las crías nazcan cuando las condicones del medio ambiente permitan su sobrevivencia (Ortavant *et al*., 1985; Bronson, 1989). Así, los periodos en los cuales se lleva a cabo la actividad de reproducción de los mamíferos varían con la duración de la gestación. En los equinos, por ejemplo, en los cuales la duración de la gestacion es de 11 meses, la actividad sexual ocurre durante la primavera, y los partos se producen durante la primavera del siguiente año (Palmer y Driancourt, 1983; Guillaume y Palmer, 1991). En cambio, en los pequeños rumiantes como los caprinos y ovinos, en los cuales la duración de la gestación es de 5 meses, la actividad sexual ocurre durante el otoño y el invierno, y los partos se producen a finales de invierno o principios de primavera (Ortavant *et al*., 1985; Gerlach y Aurich, 2000). La estacionalidad sexual se caracteriza por cambios a nivel endocrino, comportamental y gonadal, y está sincronizada por factores del medio ambiente como el fotoperiodo y las relaciones sociales, entre otros.

1.1 Hembras

1.1.1 Variaciones estacionales de la actividad sexual de las cabras y ovejas

Las hembras de razas originarias de latitudes templadas (>40º) presentan una marcada estacionalidad de su actividad sexual. En cabras de la raza Alpina, por ejemplo, la frecuencia con que ocurren las ovulaciones y el comportamiento de estro varia drásticamente de 0 % de marzo a septiembre durante el anestro estacional, a 100 % de octubre a febrero durante la estación sexual (Chemineau *et al*., 1992a). En ovejas de las razas Suffolk, Ile de France y Vendéen originarias de zonas templadas, se observan variaciones estacionales de su actividad sexual similares a las de las cabras Alpinas (Karsch *et al*., 1989; Chanvallon *et al*., 2011). La estacionalidad sexual se ha descrito también en algunas razas de cabras originarias o adaptadas a las latitudes subtropicales (Restall *et al*., 1992; Rivera *et al*., 2003; Duarte *et al*., 2008). En las cabras locales del subtrópico mexicano, particularmente las de la Comarca Lagunera, el periodo de actividad sexual inicia en agosto y termina en febrero, mientras que el periodo de anestro se observa de marzo a julio (Duarte *et al*., 2008; Figura 1). Las cabras locales de las latitudes subtropicales de Argentina o Australia presentan variaciones estacionales de su actividad sexual similares a las cabras mexicanas (Restall *et al*., 1992; Rivera *et al*., 2003). Las cabras y ovejas no gestantes presentan, durante la estación sexual, una

sucesión de ciclos estrales y ovulatorios por lo que se clasifican como especies poliestricas estacionales (Bartlewski *et al*., 2011; Fatet *et al*., 2011).

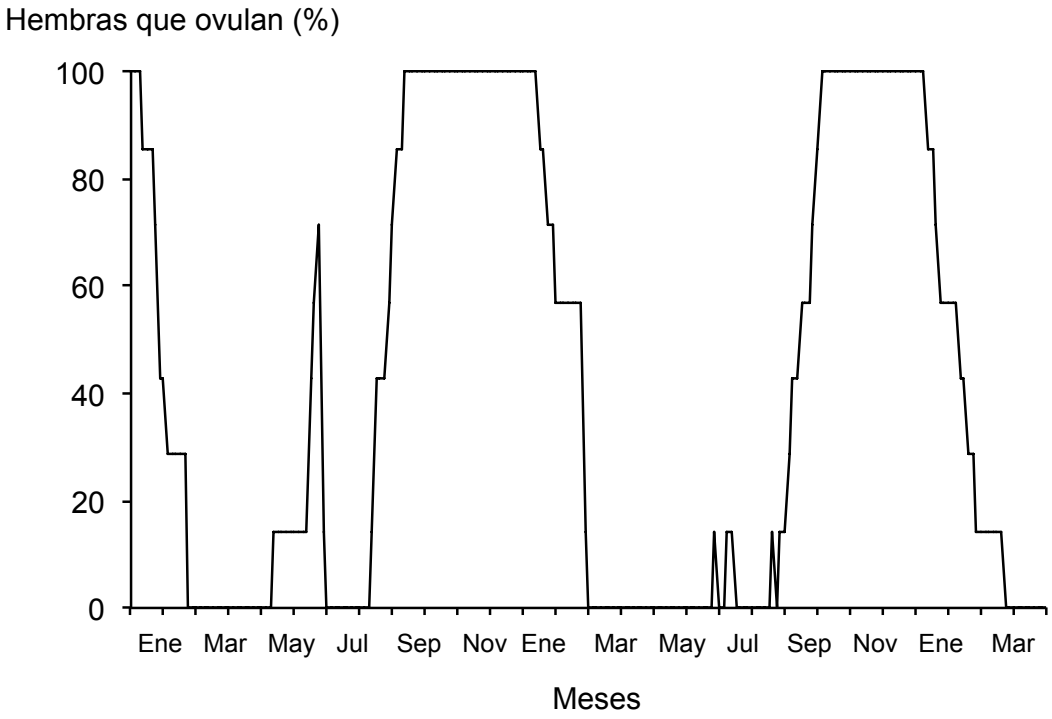

Figura 1. Variaciones estacionales de la actividad ovulatoria de cabras locales del subtrópico mexicano (26º N) sometidas a las variaciones naturales del fotoperiodo y de la temperatura ambiental (Duarte *et al*., 2008).

1.1.2 Ciclos estral y ovulatorio de cabras

El ciclo estral consiste en un conjunto de cambios endócrinos, morfológicos y comportamentales que conllevan a la expresión del comportamiento de estro, la ovulación y la preparación del tracto genital a la copulación, fertilización e implantación embrionaria. En cabras, el ciclo estral, es decir, el intervalo entre 2 expresiones sucesivas del comportamiento de estro, o el ciclo ovulatorio, es decir, el intervalo entre 2 ovulaciones sucesivas, tienen una duración promedio de 21 días (rango 17-25 días; Chemineau *et al*., 1992a). El ciclo estral, en el cual se incluye el ciclo ovulatorio, se divide en la fase folicular y la fase luteal (Fatet *et al*., 2011). La fase folicular está compuesta por 2 etapas: el proestro y el estro. El **proestro** tiene una duración promedio de 3 días, y se caracteriza por una disminución de la secreción de progesterona como consecuencia a la luteólisis o destrucción del cuerpo lúteo, y un aumento de la secreción de estradiol por los folículos. En efecto, durante esta etapa, la FSH secretada por la hipófisis, estimula el crecimiento folicular y 2 o 3 folículos son seleccionados (diámetro > 4 mmm, dominancia folicular) mientras que los otros folículos degeneran (atresia folicular). El incremento de las concentraciones periféricas de estradiol secretado por los folículos induce la receptividad sexual de las hembras, etapa denominada **estro**. Durante esta etapa que dura en promedio 3 días, el estradiol actúa por control positivo sobre el eje gonadotrópico incrementando la secreción de GnRH que a su vez induce el pico preovulatorio de LH, provocando la ovulación de 30 a 36 h después de

iniciado el estro. La fase lútea está también compuesta por 2 etapas: el metaestro y el diestro. El **metaestro** es el periodo durante el cual se forma el cuerpo lúteo a partir de las células de la granulosa. Esta etapa tiene una duración de 3 días en promedio (rango de 2-5 días) y se caracteriza por la disminución de la secreción de estradiol y un aumento de la secreción de progesterona por el cuerpo lúteo. El **diestro**, que es la etapa más larga del ciclo estral (12 días en promedio), se caracteriza por la presencia del cuerpo lúteo funcional y está asociado con niveles de progesterona altos. Finalmente, la secreción de prostaglandinas por el útero provoca la luteólisis y se levanta el control negativo operado por la progesterona sobre el eje gonadotrópico, permitiendo el inicio de un nuevo ciclo estral.

Aunque en las cabras la duración promedio del ciclo estral es de 21 días, existen también ciclos estrales y ovulatorios de corta (<17 días) y larga duración (>25 días; Chemineau *et al*., 1992a). 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 (Riera, 1982; Camp *et al*., 1983; Chemineau, 1983; Flores *et al*., 2000). En cambio, los ciclos largos son más frecuentes al final de de la estación sexual (Chemineau *et al*., 1992a). Además, puede existir una disociación estro-ovulación. Los estros sin ovulación se observan generalmente en la pubertad, al inicio de la estación sexual, al final del anestro postparto o cuando las hembras son sometidas al efecto macho (Camp *et al*., 1983; Chemineau, 1986; Jainudeen y Hafez, 1993; Mbayahaga *et al*., 1998; Flores *et al*., 2000; Lassoued y Rekik, 2005); las ovulaciones sin estro

u ovulaciones silenciosas se observan al final de la estación sexual (Chemineau *et al*., 1992a; Rivera *et al*., 2003; Lassoued y Rekik, 2005).

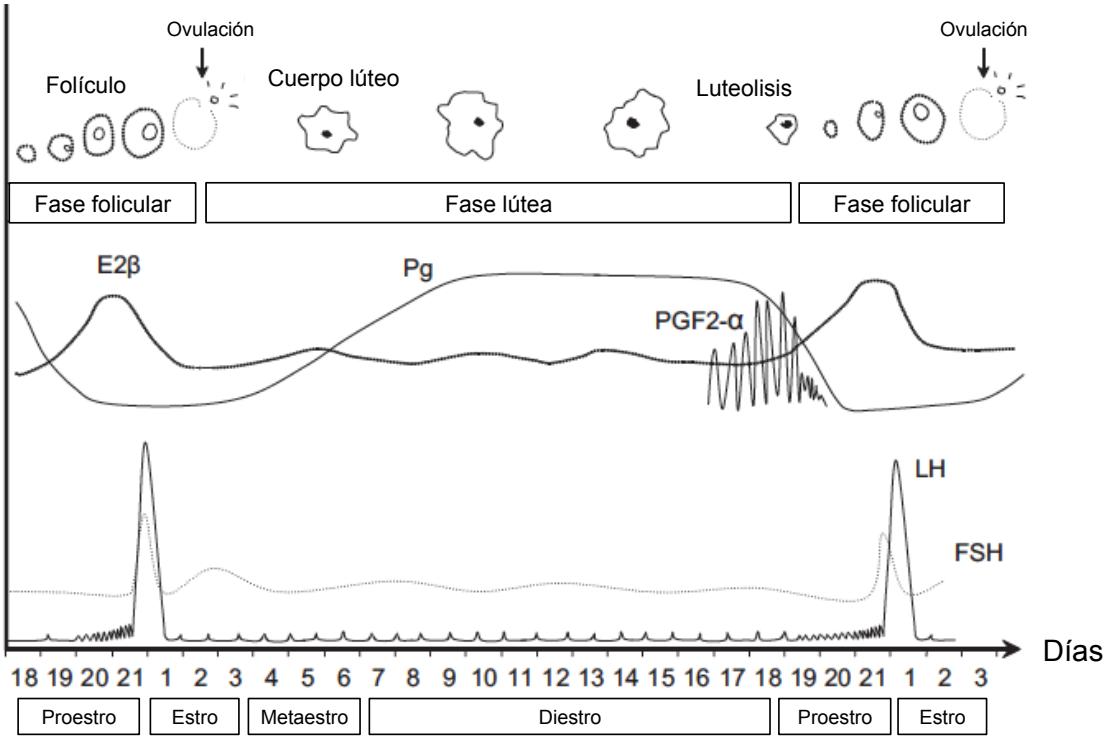

Figura 2. Representación esquemática de los eventos que ocurren durante el ciclo estral de la cabra: ciclo ovulatorio (arriba) y variaciones hormonales (abajo; modificado de Fatet *et al*., 2011).

1.2 Machos

1.2.1 Variaciones estacionales de la actividad sexual y endocrina de los machos cabríos

Los machos cabríos de razas originarias de latitudes templadas presentan una marcada estacionalidad de su actividad sexual. Los machos de raza Alpina o Saanen, por ejemplo, presentan un periodo de reposo sexual de marzo a agosto; la estación sexual se desarrolla de septiembre a febrero (Delgadillo *et al*., 1991; 1992). La estacionalidad de la actividad sexual induce cambios del peso o volumen testicular, de la producción espermática y del comportamiento sexual. Así, los machos de las razas Alpina o Saanen presentan valores más altos del peso testicular de septiembre a marzo como consecuencia de un incremento en la eficacia de la actividad espermatogénica durante este periodo (Delgadillo *et al*., 1991). El comportamiento sexual de los machos cabríos de las razas Alpina y Saanen también es modificado por las estaciones del año: la latencia a la eyaculación, es decir, el tiempo que tarda un macho en eyacular en una vagina artififcial, disminuye de septiembre a marzo, meses que corresponden a la estación sexual (Delgadillo *et al*., 1991).

En los machos de razas caprinas originarias o adaptadas a latitudes subtropicales que son estacionales, la estación sexual se presenta durante una época del año diferente a la de las razas de origen templado (Walkden-Brown *et al*., 1997; Delgadillo *et al*., 1999). En efecto, en los machos cabríos locales del

subtrópico mexicano, y particularmente los de la Comarca Lagunera, el periodo de actividad sexual inicia en mayo y termina en diciembre (Delgadillo *et al*., 1999). Como consecuencia de esta estacionalidad, el peso testicular varía de 90 g en enero-febrero durante el periodo de reposo, a 145 g en julio-agosto durante la estación sexual (Delgadillo *et al*., 1999). Asimismo, la calidad espermática determinada por la motilidad progresiva y el porcentaje de espermatozoides vivos, es mayor de mayo a diciembre que en los otros meses del año (Delgadillo *et al*., 1999). El comportamiento sexual y la intensidad del olor son también modificados por la estacionalidad sexual. En efecto, en los machos cabríos locales de la Comarca Lagunera y en los Cashmere de Australia, el comportamiento sexual determinado por la latencia a la eyaculación y el olor, son más intensos durante la estación sexual, que durante el periodo de reposo sexual (Walkden-Brown *et al*., 1994; 1997; Delgadillo *et al*., 1999; Rivas-Muñoz *et al*, 2007).

Los cambios observados en el peso testicular, el comportamiento sexual, el olor y la producción espermática, son originados por variaciones de las secreciones hormonales hipofisarias y gonadales. En efecto, en los machos cabríos de raza Alpina o Saanen las secreciones de LH y testosterona se incrementan a partir de julio-agosto, precediendo la estación sexual. Posteriormente, estas secreciones disminuyen progresivamente hasta alcanzar sus niveles más bajos en febrero (Delgadillo y Chemineau, 1992). En cambio, en los machos cabríos locales de la Comarca Lagunera, la secreción de testosterona se incrementa a partir de mayo, se mantiene elavada hasta

noviembre y disminuye en diciembre (Delgadillo *et al*., 1999). Las variaciones estacionales de las concentraciones plasmáticas de testosterona de los machos mexicanos son similares a las observadas en los machos cabríos australianos de raza Cashmere (Walkden-Brown *et al*., 1997).

1.3 Control fotoperiódico de la actividad sexual en caprinos y ovinos

Los machos y hembras de razas caprinas y ovinas originarias de latitudes templadas, así como en algunas razas originarias o adaptadas a latitudes subtropicales, el fotoperiodo es el factor ambiental principal responsable de la estacionalidad sexual. Los efectos del fotoperiodo sobre la actividad de reproducción pasan por la modificación de la secreción de melatonina por la glándula pineal. En efecto, la información fotoperiódica es percibida por la retina y transmitida por vía nerviosa a la glándula pineal (Legan y Karsch, 1983). La glándula pineal secreta principalmente melatonina: en ovinos y caprinos, los niveles plasmáticos diurnos son inferiores a 5 pg/mL, mientras que los niveles nocturnos varían de 100 a 500 pg/mL en ovinos y de 50 a 150 pg/mL en caprinos (Malpaux *et al*., 1987; Delgadillo y Chemineau, 1992). La duración de secreción de melatonina traduce la duración de la fase obscura y permite a los sistemas neuroendocrinos de los animales “medir” la duración del día (Karsch *et al*., 1984; Lincoln y Short, 1980). Al nivel del hipotálamo, la melatonina permite modular la secreción pulsátil de GnRH y, en

consecuencia, la frecuencia de secreción de LH y la actividad gonadal (Malpaux *et al*., 1998). Las variaciones del fotoperiodo modulan la retroalimentación negativa de la testosterona y del estradiol sobre el eje gonadotrópico, y es el mecanismo neuroendocrino responsable de los periodos de actividad y reposo sexual estacional en caprinos y ovinos (Pelletier y Ortavant, 1975; Karsch *et al*., 1980; Karsch *et al*., 1984; Chemineau *et al*., 1988; Duarte *et al*., 2008). En machos y hembras, la sensibilidad a la retroalimentación negativa de la testosterona y del estradiol aumenta durante los días largos (DL), lo que disminuye la secreción de GnRH y LH, provocando los periodos de reposo sexual (Pelletier y Ortavant, 1975; Karsch *et al*., 1980).

Diferentes protocolos demostraron el efecto del fotoperiodo sobre la actividad sexual de machos y hembras ovinos y caprinos. Las ovejas y carneros que se sometieron a un ciclo fotoperiódico de 6 meses, es decir, 3 meses de días crecientes y 3 meses de días decrecientes, presentaron 2 estaciones sexuales por año. La actividad sexual determinada por las ovulaciones o el tamaño testicular, se presentó durante los días cortos (DC) (Mauléon y Rougeot, 1962; Lindsay *et al*., 1984). En los machos y hembras de ovinos y caprinos que se sometieron a alternancias entre 2-3 meses de DC y 2-3 meses de DL, la actividad sexual determinada por las ovulaciones y la secreción de testosterona inició durante los DC y terminó durante los días largos DL (Bittman *et al*., 1983; Legan y Karsch, 1983; Pelletier y Almeida, 1987; Delgadillo *et al*., 2004; Duarte *et al*., 2010; Figura 3). En conjunto, estos datos sugieren que los DC estimulan y los DL inhiben la actividad sexual. Sin embargo, cuando los

animales son expuestos a DC a partir del solsticio de invierno para prolongar su actividad sexual, ésta termina al mismo tiempo que los animales expuestos al fotoperiodo natural. Esto se debe a la aparición del estado refractario o insensibilidad a los DC (Lincoln, 1980; Robinson y Karsch, 1984; Malpaux *et al*., 1988; Gómez-Brunet *et al*., 2010; Delgadillo *et al*., 2011). Asimismo, cuando los animales son expuestos a DC o DL constantes por 2 o 5 años, la actividad sexual sigue presentando variaciones. Sin embargo, estas variaciones no coinciden con las estaciones del año y están desincronizadas entre los individuos (Howles *et al*., 1982; Karsch *et al*., 1989). Esto sugiere que la estacionalidad reproductiva en los ovinos y caprinos se debe a la existencia de un ritmo endógeno de reproducción, que es sincronizado por el fotoperiodo (Howles *et al*., 1982; Karsch *et al*., 1989; Delgadillo *et al*., 2004). La percepción de los DL determina el inicio de la estación sexual, mientras que la percepción de los DC determina su duración (Malpaux *et al*., 1989; Malpaux y Karsch, 1990). La existencia de un ritmo endógeno de reproducción tiene consecuencias prácticas para el manejo de la actividad sexual a contra-estación. En efecto, el ritmo endógeno de reproducción impide inducir de forma permanente la actividad sexual de los animales al exponerlos a DC, debido a que los animales se vuelven refractarios a los DC. Por lo tanto, para evitar el establecimiento del estado refractario, e inducir la actividad sexual en los periodos de reposo sexual, los animales deben percibir alternancias entre DL y DC (Chemineau *et al*., 1992b).

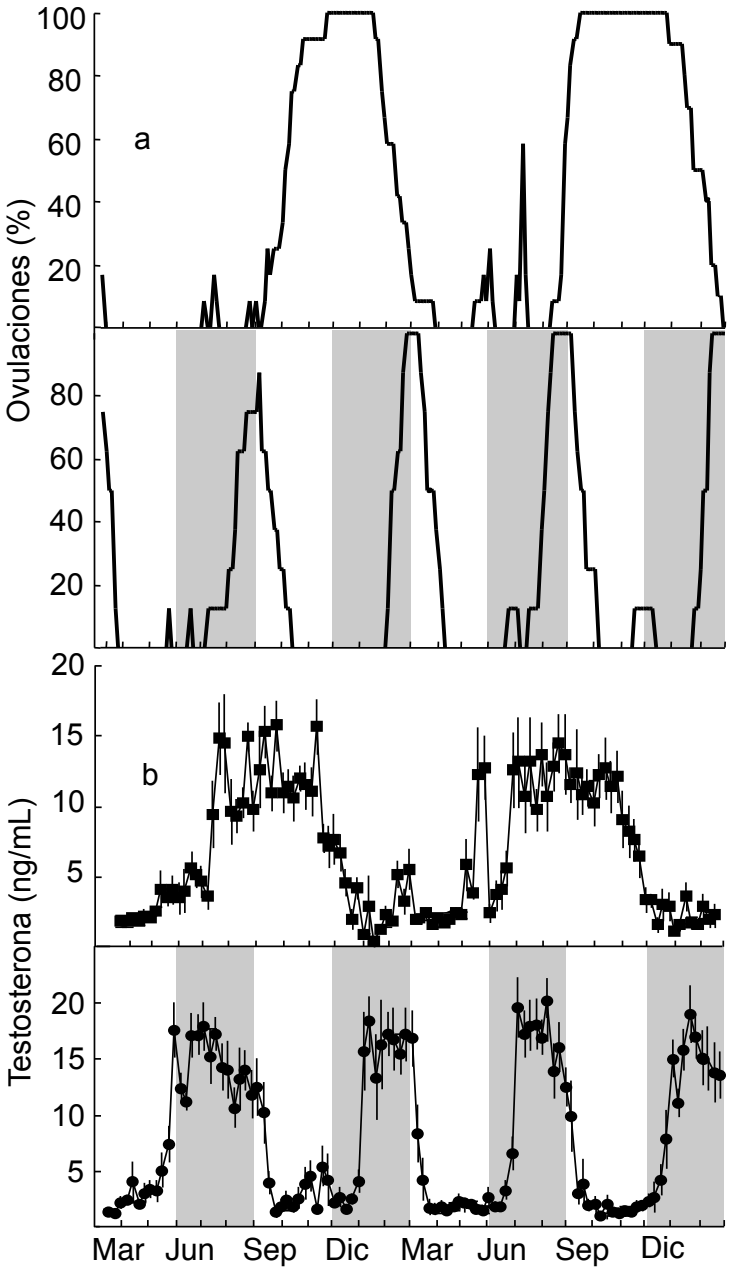

Figura 3. Variaciones de la actividad ovulatoria (a) y de las concentraciones plasmáticas de testosterona (b; promedio ± SEM) de hembras y machos cabríos locales del subtrópico mexicano (26º N) sometidos al fotoperiodo natural (arriba) o a alternancias entre 3 meses de DC y 3 meses de DL (abajo). Las áreas en gris indican los meses en que los animales fueron expuestos a DC (Delgadillo *et al*., 2004 y Duarte *et al*., 2010).

2 Tratamientos fotoperiódicos para estimular la actividad sexual de machos y hembras ovinos y caprinos

Para manipular la actividad sexual de los caprinos y ovinos, los animales deben percibir alternancias entre DL y DC para evitar la aparición del estado refractario. Dado que la estacionalidad varia de una raza a otra, los tratamientos fotoperiódicos deben aplicarse según la estación de reposo de cada una de éstas para obtener un efecto estimulatorio durante el periodo de reposo sexual natural. Los DL se proporcionan con una iluminación complementaria a la luz natural, y los días cortos se simulan aplicando implantes de melatonina o usando días cortos artificiales o el fotoperiodo natural.

2.1 Machos

En los machos ovinos y caprinos, los tratamientos fotoperiódicos basados en la sucesión de un periodo de días largos artificiales seguidos del fotoperiodo natural o de la inserción subcutánea de 2-3 implantes de melatonina, permiten inducir su actividad sexual fuera de la estación reproductiva natural. En carneros de raza Ile de France o Manech Tête Rousse originarios de latitudes templadas, la aplicación de un tratamiento fotoperiódico compuesto por 2 meses de DL artificiales (16 h de luz) a partir de enero-febrero seguidos de la inserción de 2-3 implantes subcutáneos de melatonina, estimula

la actividad sexual a partir de abril-mayo (Chemineau *et al*., 1992b; Arranz *et al*., 1995). Asimismo, en los machos cabríos de las razas Alpina o Saanen originarios de latitudes templadas, la exposición a 2-3 meses de DL 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). En los machos cabríos locales de la Comarca Lagunera, 2.5 meses de DL artificiales a partir del 1 de noviembre, seguidos del 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 (Flores *et al*, 2000; Delgadillo *et al*, 2001; 2002; Rivas-Muñoz *et al*., 2007; Figura 3). Un tratamiento similiar utilizado en los machos mexicanos, iniciando a mediados de noviembre, permite estimular la actividad sexual de los machos cabríos de la raza Payoya (Zarazaga *et al*., 2010).

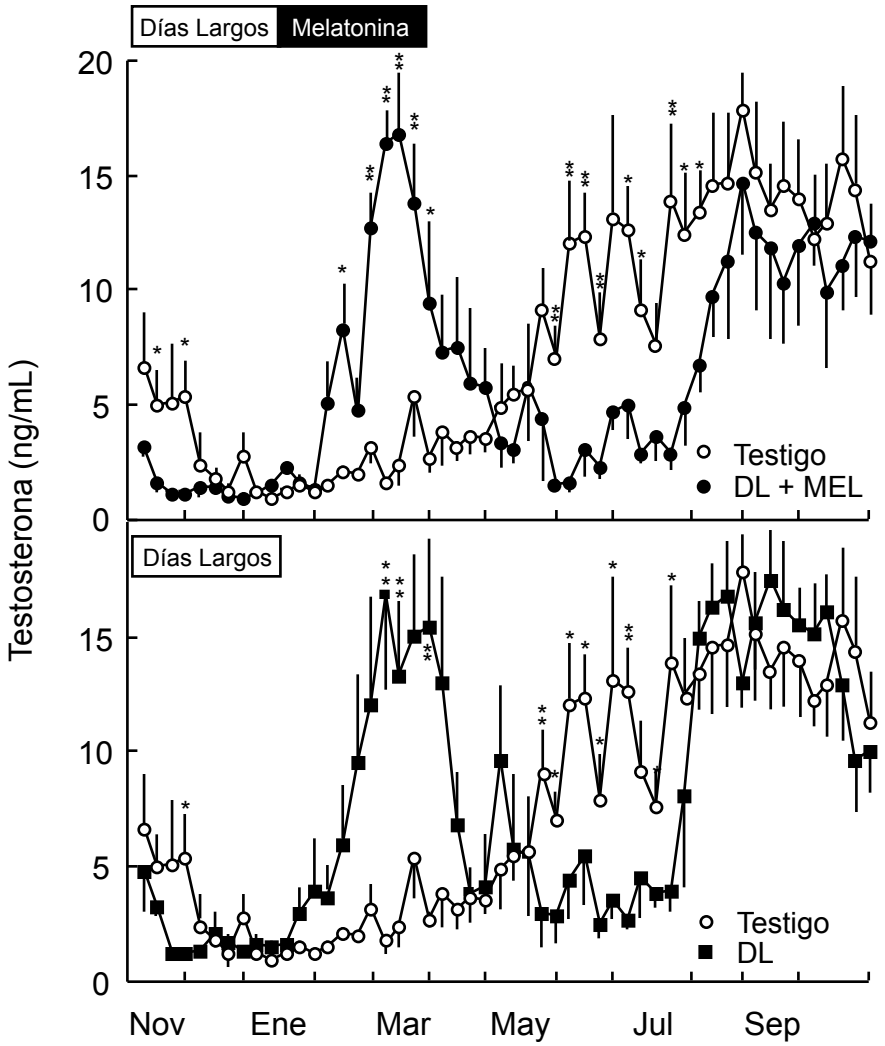

Figura 4. Variaciones de las concentraciones plasmáticas de testosterona (promedio ± SEM) en 3 grupos de machos locales del subtrópico mexicano (26ºN) sometidos a las variaciones naturales del fotoperiodo (testigo, ○), o a 2.5 meses de días largos artificiales (16 h de luz) a partir del 1 de noviembre seguidos de la aplicación de 2 implantes subcutáneos de melatonina (DL + MEL, ●) o de días naturales (DL, ■). Las muestras sanguíneas fueron obtenidas una vez a la semana (Delgadillo *et al*., 2002).

2.2 Hembras

En las hembras ovinas y caprinas de latitudes templadas, los tratamientos fotoperiódicos para estimular su actividad sexual en el anestro estacional, son similares a los aplicados en machos de estas latitudes. En estas hembras, la exposición a partir de enero a 2-3 meses de DL seguidos de 3 meses de DC artificiales, de días decrecientes o de la administración de melatonina por vía oral, subcutánea o intra-muscular, estimula la actividad sexual en mayo-junio, meses que corresponden al periodo de anestro estacional (Chemineau *et al.*, 1988; 1992b). Además, en cabras de la raza Payoya, la exposición a 3 meses de DL a partir del 17 de noviembre seguidos del fotoperiodo natural permiten inducir la actividad sexual en abril-mayo, meses que corresponden al periodo de anestro estacional (Zarazaga *et al*., 2011). Sin embargo, otra posibilidad para estimular la actividad sexual de las hembras durante el anestro estacional, es el uso de las relaciones socio sexuales, en particular el efecto macho.

3 El efecto macho

En ovinos y caprinos, la introducción de un macho en un grupo de hembras en anestro estacional puede estimular la actividad sexual. A este fenómeno se le conoce como el efecto macho (Underwood *et al*., 1944; Shelton, 1960; Chemineau, 1987; Ungerfeld *et al*., 2004).

3.1 Cambios endocrinos y conductuales inducidos por la introducción de un macho en un grupo de hembras anéstricas

La introducción de un macho cabrío o de un carnero sexualmente activo en un grupo de cabras u ovejas anéstricas puede estimular inmediatamente la frecuencia y amplitud de los pulsos de LH plasmática (respuesta a corto plazo; Figura 5; Martin *et al*., 1986; Vielma *et al*., 2009), el estro y la ovulación (respuesta a largo plazo; Poindron *et al*., 1980; Chemineau *et al*., 1986; Cohen-Tannoundji *et al*., 1986; Vielma *et al*., 2009).

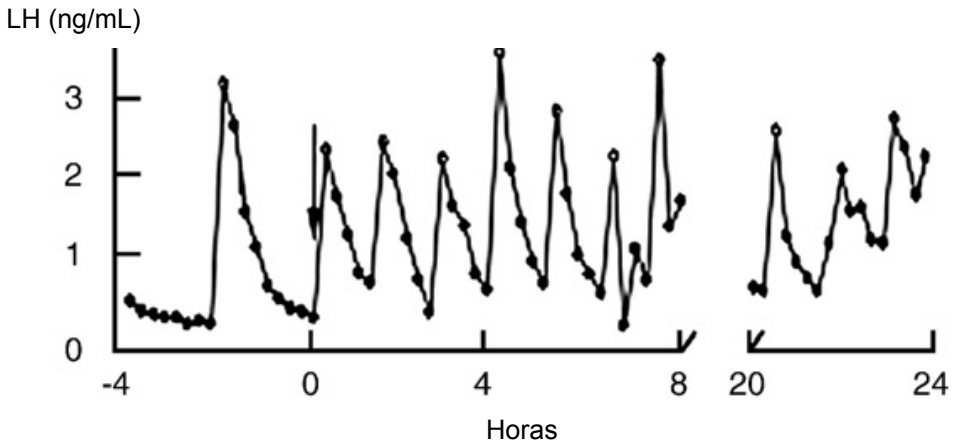

Figura 5. Perfil de secreción de LH de una cabra antes y después de ser expuesta a un macho (↓). El macho fue sometido a un tratamiento de luz del 1 de noviembre al 15 de enero para inducir un comportamiento sexual intenso durante el periodo de reposo sexual (Vielma *et al*., 2009).

En los caprinos, si el estimulo de los machos persiste, las hembras desarrollan un pico preovulatorio de LH y más del 90 % de las hembras ovulan alrededor de 50 h depués del contacto inicial con el macho (Chemineau, 1983; 1987). La primera ovulación inducida se asocia con una conducta estral en aproximadamente el 60 % de los casos (Figura 6; Chemineau, 1983; Walkden-Brown *et al*., 1993). El porcentaje de hembras gestantes después de este estro es muy bajo debido al desarrollo de un cuerpo lúteo de baja calidad (proporción de células grandes responsables de la secreción de progesterona bajo; Chemineau *et al*., 2006). En consecuencia, la mayoría de las cabras hace un ciclo ovulatorio de duración corta y vuelve a ovular en un periodo de 5 a 7 días después. Esta segunda ovulación inducida por el macho es acompañada por una conducta estral en aproximadamente 90-100 % de los casos y el cuerpo

lúteo generado es de duración normal. Por lo tanto, la mayoría de las hembras pueden quedar gestantes en esta seguda ovulación. Si no hay fecundación durante la segunda ovulación inducida, una tercera ovulación ocurre a los 21 días (Chemineau, 1987).

Fenómenos similares ocurren en ovejas (Martin et al., 1980; Poindron *et al*., 1980; Martin y Scaramuzzi, 1983). Sin embargo, contrariamente a lo observado en las cabras, la primera ovulación inducida por el macho no es acompañada de un comportamiento de estro y es clasificada como “ovulación silenciosa” (Oldham *et al*., 1978). La mitad de las hembras hacen un ciclo ovárico de duración corta y la mayoría de estas ovulaciones son también silenciosas (Oldham y Martin, 1978). El comportamiento estral aparece conjuntamente a las siguientes ovulaciones y se organiza en forma de 2 picos de actividad dentro del rebaño, aproximadamente 18 y 25 días después de la introducción del macho (Oldham y Martin, 1978).

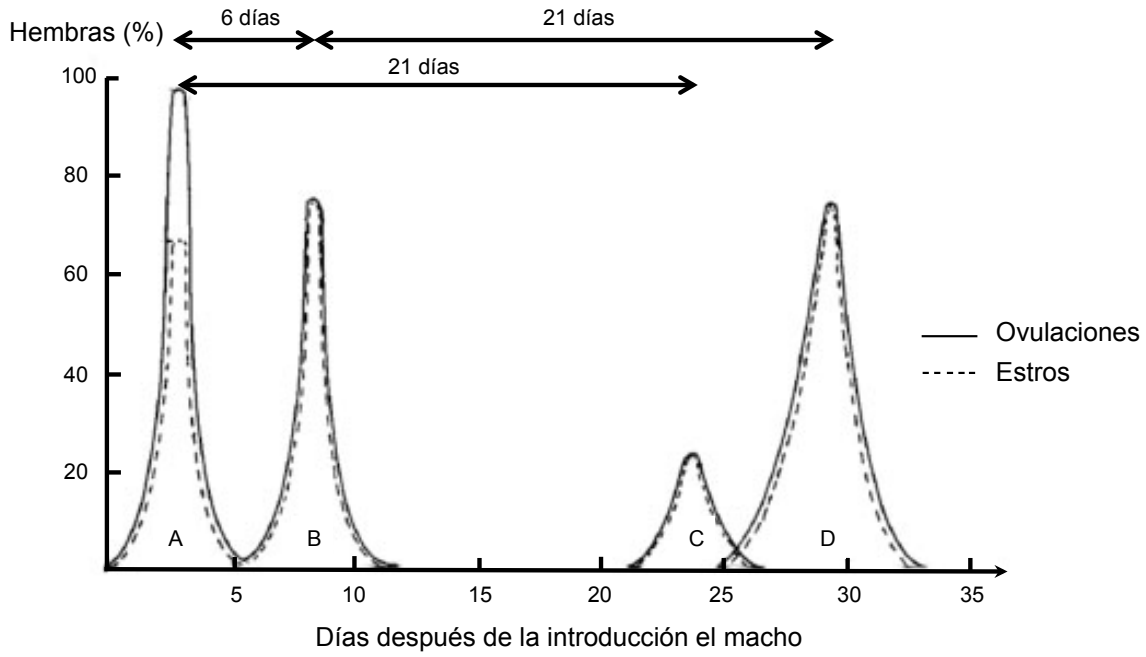

Figura 6. Representación esquemática de la respuesta al efecto macho de cabras anovulatorias. Más del 90 % de las hembras ovulan alrededor del día 3 después de la introducción de los machos (pico A). Esta primera ovulación está asociada con un comportamiento de estro en el 60 % de las hembras. La mayoría de las hembras que ovularon alrededor del día 3 experimentan un ciclo ovárico corto y ovulan nuevamente 6 días después (pico B). Si las hembras no quedan gestates, ovulan por tercera ocasión 21 días más tarde (pico D). Las otras cabras (25 %) 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).

3.2 Sentidos de las hembras implicados en la percepción del macho

El efecto macho es un fenómeno multisensorial que involucra distintas señales exteroceptivas como los olores, las vocalizaciones, los contactos físicos y visuales, así como las conductas sexuales del macho durante el cortejo sexual. Cada señal puede tener una influencia por separado, pero la mayor respuesta se obtiene cuando el macho está en contacto físico total y directo con las hembras, donde todas las señales actúan en sinergia.

3.2.1 Olfato

En los ovinos y caprinos, los machos presentan un olor intenso durante la estación reproductiva, el cual disminuye durante el periodo de reposo sexual. Estas señales olfativas parecen ocupar un papel importante en la respuesta de las hembras al efecto macho. En ovejas y cabras, varios experimentos demostraron que la exposición de las hembras al olor del macho mediante una mascara que contiene lana de carnero o pelo de macho cabrío, provoca un incremento rápido de la secreción de LH (cabras: Claus *et al*., 1990; ovejas: Cohen-Tannoudji *et al*., 1994; Gelez y Fabre-Nys, 2006). La exposición al olor del macho puede también inducir la ovulación en cabras (Walkden-Brown *et al*., 1993). En efecto, el 40 % de las cabras ovuló cuando se expusieron durante 10 días al olor del macho cabrío. Sin embargo, tanto en cabras como en ovejas, la

respuesta ovulatoria de hembras estimuladas solamente con señales olfativas es siempre más baja que cuando se exponen directamente a los machos (Shelton, 1980; Walkden-Brown *et al*.,1993). En conjunto, estos datos indican que las señales olfativas provenientes de los machos tienen un papel estimulatorio importante en las actividades endocrina y ovulatoria de las hembras expuestas al efecto macho.

3.2.2 Oido

En pequeños rumiantes, el efecto de las emisiones sonoras provenientes de los machos es controversial. Efectivamente, McComb *et al*. (1987) demostraron que las vocalizaciones de los venados reproducidas en “playback” adelantaron el inicio de la estación sexual de las hembras. Sin embargo, las vocalizaciones emitidas por los machos cabríos reproducidas en “playback” no estimularon la ovulación de las cabras en anestro estacional (Vielma *et al*., 2008). Asimismo, en ovejas se demostró que la reproducción audio-visual de machos apareandose con hembras no estimuló la secreción de LH (Hawken *et al*., 2009). Contrariamente a los resultados antes mencionados, en un estudio reciente se demostró que las vocalizaciones “in vivo” estimulan la secreción de LH y la actividad estral de cabras anéstricas (Delgadillo *et al*., 2012). En efecto, la proporción de cabras que presentaron un comportamiento de estro al ser expuestas a las vocalizaciones emitidas por los machos fue mayor que la de las

cabras aisladas. Sin embargo, no se obtuvo diferencia en la proporción de hembras que ovularon (Delgadillo *et al*., 2012). En este mismo estudio, se demostró que la respuesta de las hembras a las vocalizaciones de los machos depende de su previa experiencia sexual. De hecho, solamente las hembras que tuvieron experiencia sexual previa al estar en contacto físico total y directo con los machos, presentaron un incremento de su secreción de LH cuando fueron expuestas a las vocalizaciones emitidas por los machos (Delgadillo *et al*., 2012). En resumen, en los caprinos, las emisiones sonoras de los machos forman parte de las señales exteroceptivas que intervienen para estimular las actividades endocrina y estral de las hembras expuestas al efecto macho.

3.2.3 Tacto

El contacto directo entre los machos y las hembras también está involucrado en la respuesta de las hembras expuestas al efecto macho. La mayor respuesta ovulatoria de las hembras se obtiene cuando están en contacto directo con los machos. En efecto, en ovinos y caprinos se demostró que la separación de los sexos por una cerca que permite a las hembras percibir las señales olfativas, auditivas y visuales de los machos, reduce la proporción de hembras que ovulan en comparación con las que están en contacto físico completo con los machos (Shelton, 1980; Pearce y Oldham, 1988). Asimismo, la proporción de cabras que ovulan es mayor cuando están en contacto directo con los machos que cuando están separadas por un pasillo

(88 % vs. 15 %; Chemineau, 1987). Finalmente, en ovinos se demostró la importancia de las señales táctiles al utilizar hembras a las cuales se les retiró los bulbos olfatorios, es decir, privadas del sentido del olfato (Cohen-Tannoudji *et al*., 1986). En efecto, en hembras bulbectomizadas, la secreción de LH se incrementó de manera similar que en las hembras intactas cuando fueron expuestas a carneros enteros, pero fue inferior cuando se expusieron a la lana de ellos (Cohen-Tannoudji *et al*., 1986). Sin embargo, es probable que otras señales provenientes del macho como las vocalizaciones pudieron también intervenir en el incremento de la secreción de LH (Delgadillo et al., 2012). En resumen, estos datos indican que las interracciones táctiles entre machos y hembras forman parte de las señales exteroceptivas que participan en la estimulación endocrina y sexual de las hembras expuestas al efecto macho.

3.2.4 Vista

El contacto visual entre machos y hembras también puede influir sobre la respuesta de las hembras expuestas al efecto macho. En efecto, cuando se agregaron las señales visuales al olor y a las vocalizaciones de los machos, la proporción de cabras que ovuló se incrementó del 20 % al 41 % (Shelton, 1980). En ovejas, se mostró que la exposición a imágenes de caras de carneros provoca la liberación de noradrenalina al nivel del hipotálamo mediobasal (Fabre-Nys *et al*., 1997). La secreción de noradrenalina en esta región, que está

involucrada en la respuesta sexual de las hembras al efecto macho, sugiere que las señales visuales pueden estimular la actividad sexual de las hembras. Además, Hawken *et al*. (2009) observaron que en las ovejas expuestas a imágenes proyectadas de machos se incrementó la secreción de LH. Sin embargo, cuando otras hembras fueron expuestas a un video de machos (estímulos visual y auditivo) no se observó ningún cambio en la secreción de LH. En conjunto, estos resultados sugieren que en algunas condiciones experimentales, las señales visuales desempeñan un papel importante para estimular la actividad endocrina de las hembras expuestas al efecto macho.

3.3 Factores que afectan la respuesta estral y ovulatoria de las hembras expuestas al efecto macho

En ovejas y cabras, la respuesta al efecto macho puede ser influenciada por varios factores como la intensidad del comportamiento sexual del macho, la proporción macho:hembra y la duración del contacto entre machos y hembras, entre otros (Álvarez-Ramírez *et al*., 2001; Delgadillo *et al*., 2006; 2009).

3.3.1 Intensidad del comportamiento sexual del macho

En ovejas y cabras se demostró que la intensidad del comportamiento sexual de los machos es un elemento clave para estimular el estro y la ovulación en la mayoría de las hembras expuestas a los machos (Signoret *et al*., 1982; Perkins y Fitzgerald, 1994; Flores *et al*., 2000; Delgadillo *et al*., 2002). En efecto, en carneros castrados y tratados con andrógenos, los machos que mostraron un comportamiento sexual más intenso fueron más eficaces para inducir la ovulación en las ovejas (Signoret *et al*., 1982). En otro experimento se demostró que los carneros intactos que exhibieron un comportamiento sexual intenso, fueron capaces de estimular la ovulación en una proporción mayor de ovejas que los machos con un comportamiento sexual más débil (95 % vs 78 %, respectivamente; Perkins y Fitzgerald, 1994).

En las cabras que manifiestan una marcada estacionalidad reproductiva, la proporción de hembras que ovula al ser expuestas al efecto macho a la mitad del anestro, es muy baja y en muchas ocasiones no hay respuesta ovulatoria de las hembras. Aunque no se sabe la razón por la cual las hembras no responden al efecto macho, es probable que sea porque los machos que están en reposo sexual, despliegan un comportamiento sexual débil. Esta hipótesis se comprobó al utilizar machos cabríos foto-estimulados. Así, en las cabras de la Comarca Lagunera, el 100 % de las hembras presentaron una actividad estral y ovulatoria al ser expuestas durante el anestro estacional a machos inducidos a una intensa actividad sexual al someterlos a 2.5 meses de DL artificiales a partir

del 1 de noviembre seguidos o no de la aplicación de 2 implantes subcutáneos de melatonina (Flores *et al*., 2000; Delgadillo *et al*., 2002; Rivas-Muñoz *et al*., 2007; Fitz-Rodríguez *et al*., 2009). En cambio, ninguna de las cabras ovularon cuando se expusieron a machos no tratados que estaban en reposo sexual (Flores *et al*., 2000; Delgadillo *et al*., 2002). Estos resultados se deben probablemente al intenso comportamiento sexual desplegado por los machos sometidos a los tratamientos fotoperiódicos. En efecto, en las cabras expuestas a machos foto-estimulados que desplegaban un intenso comportamiento sexual, la pulsatilidad de LH se mantuvo elevada durante 24 h después de la introducción de machos. En cambio, en las cabras expuestas a machos foto-estimulados sedados para evitar que desplegaran su comportamiento sexual, la pulsatilidad disminuyó 4 h después del primer contacto entre machos y hembras (Vielma *et al*., 2009). Es interesante señalar que el estudio con machos foto-estimulados despiertos y sedados se repitió, pero el periodo de contacto entre machos y hembras se prolongó por 4 días (Martínez-Alfaro *et al*., 2011). En esas condiciones, la proporción de hembras que ovularon fue mayor en las expuestas a los machos despiertos que aquellas en contacto con los machos sedados (90 vs 0 %, respectivamente; Martínez-Alfaro *et al*., 2011). En conjunto, estos datos demuestran que la respuesta de las cabras expuestas a los machos depende de la intensidad del comportamiento sexual de éstos. El tratamiento fotoperiódico mejora el comportamiento sexual de los machos, y en consecuencia, la eficacia de éstos para estimular la actividad sexual de las cabras anéstricas.

3.3.2 Proporción macho:hembra

En ovejas y cabras se demostró que la proporción macho:hembra es un factor que puede modificar la respuesta de las hembras expuestas al efecto macho. En ovejas de la raza Merino, Lindsay *et al*. (1992) observaron menos hembras en estro cuando se utilizó una proporción macho:hembra de 1 % que cuando fue de 3 o 6 %. En ovejas de la raza Corriedale, una disminución de la proporción macho:hembra de 16 a 8 % reduce el número de hembras que presentaron un comportamiento de estro, pero las proporciones de hembras que ovularon no fueron diferentes (Rodríguez-Iglesias *et al*., 1997). En cabras expuestas a machos foto-estimulados, la proporción de hembras que presentaron un comportamiento estral no se redujo al disminuir la proporción macho:hembra a 10, 5 o 3 % (Carillo *et al*., 2007). Sin embargo, esta disminución de la proporción macho:hembra aumentó el intervalo entre la introducción de los machos y el inicio del estro. En resumen, estos resultados sugieren que la respuesta ovulatoria y estral de las hembras puede ser modificada por la proporción macho:hembra. En los experimentos realizados en la Comarca Lagunera con machos foto-estimulados, la proporción macho:hembra de alrededor 10 % asegura que una proporción elevada (>80 %) de hembras sea fertilizada al usar el efecto macho (Flores *et al*., 2000; Delgadillo *et al*., 2002; Luna-Orozco *et al*., 2008).

3.3.3 Duración del contacto entre machos y hembras

La duración del contacto entre machos y hembras es otro factor que modifica la respuesta sexual de las hembras expuestas a los machos. En efecto, el contacto entre los sexos debe ser mantenido durante varios días para estimular la actividad ovulatoria en la mayoría de las hembras (Oldham y Pearce, 1983). En ovejas, por ejemplo, solo el 18 % de las hembras ovularon cuando se expusieron a los machos por 24 h, mientras que el 53 y el 61 % de ellas ovularon cuando estuvieron en contacto con los machos por 4 o 13 días, respectivamente (Signoret *et al*., 1982). Asimismo, en las cabras de la raza Cashemere, solamente el 24 % de las hembras ovularon cuando se expusieron 16 h por día a machos durante 10 días (Walkden-Brown *et al*., 1993). Estos resultados se deben, probablemente, a que en las cabras como en las ovejas, la exposición a machos induce una rápida activación de la secreción de LH (respuesta a corto plazo), pero la estimulación de la ovulación y del comportamiento de estro requiere más tiempo de contacto entre los sexos (respuesta a largo plazo; Signoret *et al*., 1982; Ungerfeld et al., 2004; Vielma *et al*., 2009). En efecto, en ovejas se demostró que la secreción de la LH se incrementa cuando las hembras se ponen en contacto con los machos. Sin embargo, cuando se retiran los machos del grupo de hembras, la secreción de LH disminuye y vuelve al nivel que existía antes de la introducción de los machos, lo que evita que se produzca la ovulación (Oldham y Pearce, 1983). Sin embargo, Rivas-Muñoz *et al*. (2007) reportaron que en las cabras locales

del subtrópico mexicano, la reducción del tiempo de contacto con machos cabríos foto-estimulados de 24 h a 16 h por día, no disminuyó la respuesta estral de las hembras expuestas al efecto macho durante 18 días. En este experimento, la proporción de hembras detectadas en estro fue alta (> 90 %) y similar entre las hembras expuestas continuamente (24 h por día) o discontinuamente (16 h por día) a los machos (Rivas-Muñoz *et al*., 2007). Estos resultados obtenidos con cabras del subtrópico mexicano demuestran que 16 h de contacto diario con machos cabríos foto-estimulados, son suficientes para inducir la ovulación en la mayoría de las cabras expuestas a los machos. Sin embargo, no se sabe si el tiempo de contacto entre machos cabríos foto-stimulados y cabras anéstricas se puede reducir aun más, sin disminuir las respuestas sexual y reproductiva de las hembras. Por tal motivo, se efectuaron tres estudios para determinar si la duración de contacto entre cabras y machos cabríos foto-estimulados se puede reducir sin afectar las respuestas sexual y reproductiva de las hembras anéstricas.

OBJETIVOS

1. Determinar si los machos foto-estimulados son capaces de estimular la actividad sexual de cabras anovulatorias al estar en contacto con ellas menos de 16 h diarias durante 15 días consecutivos.

2. Determinar si los machos foto-estimulados son capaces de estimular la actividad sexual de cabras anovulatorias al estar en contacto con ellas menos de 4 h diarias durante 15 días consecutivos.

3. Determinar si los machos foto-estimulados son capaces de estimular la actividad sexual de 3 grupos diferentes de hembras anovulatorias al estar en contacto con ellas 4 h diarias durante 15 días consecutivos.

HIPÓTESIS

1. Los machos foto-estimulados son capaces de estimular la actividad sexual de cabras anovulatorias al estar en contacto con ellas menos de 16 h diarias durante 15 días consecutivos.

2. Los machos foto-estimulados son capaces de estimular la actividad sexual de cabras anovulatorias al estar en contacto con ellas menos de 4 h diarias durante 15 días consecutivos.

3. Los machos foto-estimulados son capaces de estimular la actividad sexual de 3 grupos diferentes de hembras anovulatorias al estar en contacto con ellas 4 h diarias durante 15 días consecutivos.

ARTÍCULOS

Artículo 1. Four hours of daily contact with sexually active males is sufficient to induce fertile ovulation in anestrous goats.

Publicado: Hormones and Behavior 58 (2010) 473-477.

Artículo 2. One hour of daily contact with sexually active males is sufficient to induce fertile ovulation in anestrous goats.

Artículo 3. Sexually active bucks are able to stimulate three successive groups of females per day with a 4-hour period of contact.

Publicado: Physiology and Behavior 106 (2012) 259-263.

1 Four hours of daily contact with sexually active males is sufficient to induce fertile ovulation in anestrous goats

Hormones and Behavior 58 (2010) 473–477

Contents lists available at ScienceDirect

Hormones and Behavior

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

Four hours of daily contact with sexually active males is sufficient to induce fertile ovulation in anestrous goats

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ARTICLE INFO

Article history:
Received 1 March 2010
Revised 19 April 2010
Accepted 3 May 2010
Available online 10 May 2010

Keywords:
Male effect
Anovulatory does
Duration of contact
Ovulation
Olfaction

ABSTRACT

The study was conducted on two consecutive years to determine whether ovulatory activity can be induced in anovulatory goats by exposing them to sexually active bucks for 4, 8, 12 or 16 h per day during 15 consecutive days. In experiment 1, females remained continuously in the experimental pens where they were in contact with males. One group remained isolated from males (controls) and four other groups were exposed to sexually active males for 4, 8, 12 or 16 h per day. In experiment 2, females were taken away to "resting" pens free of male odours between the periods of contact with bucks. They were allocated to 5 groups as in experiment 1. Ovulations were determined by progesterone plasma levels and transrectal ultrasonography. Pregnancy was determined by abdominal ultrasonography. In both experiments, more than 90% of females exposed to the bucks had at least one ovulation during the whole experiment whereas only 11 or 0% (experiments 1 and 2, respectively) did so in the control group ($P<0.001$). Furthermore, the proportion of females ovulating did not differ among groups depending on duration of contact with bucks ($P>0.05$). In both experiments, pregnancy rates were not affected by the daily duration of contact with males ($P>0.05$). To conclude, 4 h of daily contact with sexually active males is sufficient to stimulate ovulatory activity in anovulatory goats and this effect is not due to the presence of olfactory cues from the males remaining in the pens.

Introduction

In small ruminants such as sheep and goats, the introduction of a male into a group of anovulatory females can induce their sexual activity within a few days. This phenomenon, known as the "male effect" or teasing, has been extensively studied in breeds of ewes (Underwood et al., 1944; Signoret, 1980; Martin et al., 1986) and goats that show a period of seasonal anestrus (Shelton, 1960; Chemineau et al., 2006; Delgadillo et al., 2009). Many factors influence the response of females to the male effect, among which the duration of contact between sexes, the olfactory cues provided by the male, and the intensity of its sexual behaviour are particularly important.

It was shown that the contact between males and females must be maintained for several days to stimulate the ovulatory activity in most females (Oldham and Pearce, 1983). Indeed, in both goats and ewes, the first exposure to males induces a rapid activation of LH secretion (short-term response), but the stimulation of ovulation and estrous behaviour needs a larger time of contact between the 2 sexes (long-term response, Signoret et al., 1982; Ungerfeld et al., 2004; Vielma et al., 2009). In fact, in ewes it was shown that when males were removed from a group of females, LH secretion decreased to its initial level 2 h after males' withdrawal and failed to lead to ovulation (Oldham and Pearce, 1983). In another study in sheep, only 18% of ewes ovulated when exposed to males for 24 h, whereas 51% ovulated when the males were maintained for 4 days and 61% if males were maintained 15 days (Signoret et al., 1982). In cashmere goats, only 24% of females ovulated when exposed 16 h per day to bucks during 10 days (Walkden-Brown et al., 1993a). However, Rivas-Muñoz et al. (2007) found that in local goats from subtropical Mexico, reducing contact with bucks from 24 h to 16 h per day does not affect the response of females submitted to a male effect during 18 days. Indeed, in this experiment, occurrence of estrous behaviour and mean interval between introduction of males and onset of estrous behaviour were similar between females exposed continuously or discontinuously to males (96% vs. 92% and 3.9 ± 0.6 days vs. 2.8 ± 0.4 days, respectively; Rivas-Muñoz et al., 2007). A likely explanation for the difference between these two studies is that long-days-treated—and therefore sexually active—bucks were used by Rivas-Muñoz et al. (2007), whereas Walkden-Brown et al. (1993a) used males that had not been treated with light and which were therefore probably either in sexual rest or with a low sexual behaviour.

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0018-506X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2010.05.002

Whether sexually active bucks can induce ovulation and sexual activity in anestrous goats, when contact between sexes is reduced to less than 16 h/day remains an open question. Indeed, in addition to its sexual activity, the male provides also the female with olfactory cues that are determinant in triggering the neuroendocrine response that leads to ovulation. This has been well established in sheep and goats (Walkden-Brown et al., 1999; Claus et al., 1990; Cohen-Tannoudji et al., 1994). Many experiments in both species showed that exposure to male odour alone (using hair or fleece) may induce an ovulatory response in seasonally anovulatory goats (Chemineau et al., 1986; Claus et al., 1990; Walkden-Brown et al., 1993a) and ewes (Knight and Lynch, 1980; Cohen-Tannoudji et al., 1994; Gelez and Fabre-Nys, 2006). However, in both species, the ovulatory response obtained in females stimulated with olfactory cues alone is always lower than that of females exposed to males (Shelton, 1980; Walkden-Brown et al., 1993a). A recent study in the goat has shown that while olfactory cues from the male are sufficient to trigger the initial secretion of LH in the female, male sexual behaviour appears necessary for LH secretion to be maintained so that ovulation can occur (Vielma et al., 2009). In fact, it may be the combination of olfactory cues with male sexual behaviour that ensures the success of the male effect in goats stimulated by light-treated bucks. Indeed, such a combined effect of olfactory cues and sexual behaviour of the buck could explain the high response reported by Rivas-Muñoz et al. (2007) in goats experiencing only 16 h of daily contact with sexually active bucks. The bucks may have contaminated the pen in which the females were maintained with male olfactory cues during their daily presence by faeces, urine and rubbing to the fence posts of the enclosure. Then, the continuous presence of these cues may have compensated for the discontinuous presence of the males. If so, even shorter durations of contact between anovulatory goats and active bucks may well lead to a high percentage of females responding to the male effect when females remain in the same pen all the time. In contrast, if females are taken away to pens free of olfactory cues from males, it could be expected that shorter durations of daily contact will lead to significantly reduced percentage of females responding to the presence of active bucks. To test this possibility, we exposed seasonally anovulatory females for 16, 12, 8 or 4 h daily to sexually active bucks with does remaining or not in the pens where they were in contact with males.

Materials and methods

General conditions

Two experiments were carried out in two consecutive years during the non-breeding season. In both experiments, the male effect was performed over a period of 15 days in March, using males that had been rendered sexually active by a photoperiod treatment of artificial long days. In experiment 1, females remained in the pens of stimulation during the whole experiment and males were removed to a distant pen after the daily duration of contact ended. In experiment 2, females were removed from the pens of stimulation to distant pens after the daily duration of contact ended and males remained in the pens of stimulation throughout the experiment. The experiments were performed using local goats (*Capra hircus*) from the Laguna region in the State of Coahuila, Mexico (latitude, 26°23′N and longitude, 104°47′W). In females, non-breeding season lasts from March to August, and in bucks from January to April (Delgadillo et al., 1999; Duarte et al., 2008). All females had given birth between October and December of the year before the studies were carried out, and were milked manually once daily during the studies. Females and males were kept in open shaded pens (6×4 m), and fed 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate (14% CP; 1.7 Mcal/kg) with free access to water during the studies.

The animals were maintained under good management conditions that fulfilled the nutritional requirements of the animals and the procedures used in the experiments were in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Sexual activation of bucks—photoperiodic treatment

In both experiments, eight bucks were kept together in an open pen (6×6 m). The males were subjected to a treatment of long days (16 h of light/8 h of darkness) from November 1st to January 15th. On January 16th, the light treatment was stopped and the bucks were exposed to natural variations of day-length until the end of the study. This treatment has previously been shown to take a further 45 to 60 days to produce a stimulatory effect on male reproductive activity. Testosterone secretion is stimulated from the end of February to the end of April and, as a consequence, the production of sexual odour and the sexual behaviour of bucks are improved during these months corresponding normally to the non-breeding season (Delgadillo et al., 2002; Rivas-Muñoz et al., 2007). For this reason, light-treated males are used in March–April. Moreover, it was found in both goats (Delgadillo et al., 2002) and ewes (Perkins and Fitzgerald, 1994) that a high sexual activity of males is a key component to obtain a high ovulatory response of females to the male effect. From the 8 bucks rendered sexually active by light treatment, four pairs of males were made at random. Not all the bucks used in experiment 1 were used in experiment 2, therefore pairs of males were different in experiments 1 and 2. On March 10th and 18th (experiments 1 and 2, respectively), bucks were individually submitted to behavioural tests in order to confirm their high sexual activity before beginning the experiment. Every buck was exposed to an anestrous female goat and the following behaviours were recorded: self urination, flehmen, ano-genital sniffing, nudging, mounting attempts and mounts (with or without vaginal intromission; Gonzalez et al., 1988; Fabre-Nys, 2000). The quality of semen was assessed in undiluted semen by determining the progressive sperm motility and percentage of live spermatozoa observed immediately after semen collection (Delgadillo et al. , 1992). Semen was collected using an artificial vagina, when males were presented to an intact estrus-induced doe. All males had good quality semen with more than 70% of live spermatozoa and a sperm motility higher than 3 (Delgadillo et al., 1992), and the average sexual behaviour and quality of semen were similar between the four pairs of bucks.

Preparation of females

In experiment 1 (year 1), 71 multiparous anovulatory female goats were used. On March 1st and 8th, each female was submitted to a transrectal ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe in order to determine their ovarian cyclicity. On March 10th, females were divided into 5 groups balanced for body weight and kept in open pens (6×4 m): the control group ($n=9$; 42.8±1.6 kg: mean±SEM) was isolated from males. The other four groups were exposed daily to sexually active males for 4 ($n=15$; 42.7±1.4 kg), 8 ($n=15$; 45.6±1.4 kg), 12 ($n=15$; 41.9±1.2 kg) or 16 h ($n=17$; 41.3±1.0 kg).

In experiment 2 (year 2), females from the same flock were tested for ovarian cyclicity by transrectal ultrasonography and 84 anovulatory multiparous local goats were divided in 5 groups. As in experiment 1, groups were balanced for body weight, but otherwise females were allocated to each group at random. The control group ($n=12$; 40.7±1.0 kg) was totally isolated from males. The other four groups ($n=18$ each) were exposed daily to sexually active males for 4 (40.7±1.3 kg), 8 (40.6±1.2 kg), 12 (40.8±1.1 kg) or 16 h (40.6±1.1 kg). Females were kept in the same conditions as in experiment 1.

Male effect

On March 12th and 27th (experiments 1 and 2, respectively; day 0), females of each experimental group were exposed to one of the 4 pairs of bucks treated with long days ($n=2$/group), selected at random. In both experiments, bucks were placed with females for 15 days and the pairs of bucks were changed between experimental groups every day.

In experiment 1, bucks were introduced each day at 08:00. In groups of does exposed to males for 4, 8, 12 and 16 h, bucks were removed from pens at 12:00, 16:00, 20:00 and 24:00 h respectively, and then placed until next day in another pen located at more than 200 m from the experimental pens. In addition, the distance between the 5 groups was more than 100 m, thus preventing any risk of interference by the treatments between groups (Walkden-Brown et al., 1993a).

In experiment 2, groups of does exposed to males for 4, 8, 12 and 16 h were introduced each day at 08:00 and removed from pens with males at 12:00, 16:00, 20:00 and 24:00 h respectively. Each group of females was placed until next day in separated holding pens that had not contained bucks; these holding pens were located at more than 200 m from experimental pens. In addition, as in experiment 1 the distance between experimental groups was of at least 100 m.

Measurements

In experiment 1, the first and second male-induced ovulations and ovulation rate were assessed by the presence and number of corpora lutea observed in each female by transrectal ultrasonography 6 and 20 days after introduction of the bucks. In addition, daily blood samples were taken from days 3 to 9 and on day 19 after exposure to males in order to confirm the presence of corpora lutea assessed by ultrasonography. The percentage of short and normal cycles was inferred from plasma concentrations of progesterone. In this experiment the presence of corpora lutea was confirmed by progesterone levels. Therefore, in experiment 2, the first and second male-induced ovulations and ovulation rate were only assessed by transrectal ultrasonography as described in experiment 1. Transrectal ultrasonography was performed using an Aloka SSD-500 machine connected to a 7.5 MHz linear probe. In both experiments, pregnancy rates (pregnant does/does exposed to males) were determined by abdominal ultrasonography 52 days after exposure to males using the same machine connected to a 3.5 MHz abdominal probe. Fertility (number of females kidding/number of females exposed to males) and prolificacy (number of kids born/number of females giving birth) were determined at parturition.

Blood sampling

Blood samples were collected by jugular venipuncture in tubes containing heparin. Plasma was obtained after centrifugation at 3500×*g* for 30 min and stored at −20 °C until hormone concentrations were measured. Concentrations of plasma progesterone were measured by RIA in duplicate as described by Saumade et al. (1985). Sensitivity was 0.1 ng/mL. The intra- and inter-assay coefficients of variation were 5 and 7% respectively. Females in which progesterone increased (≥0.5 ng/mL) and then decreased to basal levels during the first 7 days after male exposure and increased again were classified as having a short ovulatory cycle. Females in which progesterone always increased during the first 7 days after male exposure were classified as having a normal ovulatory cycle. Females with progesterone concentrations ≥0.5 ng/mL were considered to have ovulated (Chemineau et al., 2006).

Statistical analyses

Ovulation rates and prolificacy were all compared using the Kruskall–Wallis-test followed by the Mann–Whitney *U*-test. The proportions of females having ovulated, the proportions of does displaying short or normal ovulatory cycles, pregnancy rates and fertility were all compared between groups using the Fisher–Freeman–Halton exact probability test for multiple-group comparisons and Fisher exact probability test for two-group comparisons. Data are reported as mean ± standard error of the mean. Analyses were computed using SYSTAT 10 (Evenston, ILL, USA, 2000) or StatXact 6 (Cytel Software, Cambridge, MA).

Results

Response of females to the male effect: ovarian activity and fertility

In both experiments, more than 90% of females exposed to the sexually active bucks had at least one ovulation during the whole experiment whereas only 11 or 0% (experiments 1 and 2, respectively) did so in the control group ($P<0.001$, Tables 1 and 2). Furthermore, the proportion of females that ovulated did not differ among groups of does exposed daily for 4, 8, 12 or 16 h to the sexually active males ($P=1$; Tables 1 and 2). In groups exposed to males in both experiments, the ovulation rate of the second male-induced ovulation and the pregnancy rates did not differ significantly depending on the daily duration of contact with males ($P\geq0.41$ and $P\geq0.50$; Tables 1 and 2). In experiment 1, the proportions of females exposed to bucks that displayed short and normal ovulatory cycles did not differ among groups ($P=0.13$; Table 1). In experiment 2, fertility and prolificacy were not affected by the daily duration of contact with males ($P=0.99$ and $P=0.12$; Table 2).

Discussion

The high ovulatory response of goats exposed intermittently to males, both when they remained in the experimental pen (experiment 1) or when they were shifted to another resting pen free of residual odour from bucks (experiment 2), does not support our hypothesis that contamination of the pen by male odour is crucial to obtain a high ovulatory response in anovulatory goats exposed intermittently to males. In addition, no differences were found depending on the duration of daily contact with males: the proportion of females ovulating exceeded 90% and the proportion of females displaying short ovulatory cycles, pregnancy rate and fertility were similar regardless of duration of contact with the males, even when duration of contact with males lasted only 4 h daily. Besides, the ovulatory response and the percentages of pregnant does observed in the present studies are similar to those reported in previous studies in which females were continuously exposed to sexually active males (Flores et al., 2000; Delgadillo et al., 2002). Moreover, a daily contact of 4 h between males and females provided the same response as 16 h, whether females remained in experimental pens or not. This

Table 1
Ovulatory responses and pregnancy rate of anestrous female goats exposed for 4, 8, 12 or 16 h to males rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th. The period of contact between males and females was ended each day by the removal of males from the experimental pens; females remained in the same pens throughout the experiment.

Groups	*n*	Females with ovulations (%)	Ovulation rate (mean ± SEM)	Females with short ovarian cycles (%)	Females with normal ovarian cycles (%)	Pregnancy rate[1] (%)
Isolated	9	11.1[a]	–	–	–	–
4 h	15	100[b]	1.9±0.18[a]	86.7[a]	13.3[a]	93.3[a]
8 h	15	100[b]	1.8±0.14[a]	86.7[a]	13.3[a]	86.7[a]
12 h	15	100[b]	1.5±0.14[a]	60[a]	33.3[a]	100[a]
16 h	17	94.1[b]	1.6±0.13[a]	58.8[a]	35.3[a]	94.1[a]

[a,b]Values with different letters within each column are different ($P<0.001$).
[1] Percentage of pregnant females detected as pregnant by ultrasonography 52 days after the introduction of males.

Table 2
Ovulatory response, pregnancy rate, fertility and prolificacy of anestrous goats exposed for 4, 8, 12 or 16 h to males rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th. The period of contact between males and females was ended each day by moving the females from the experimental pens to resting pens located 200 m away; males remained in the experimental pens throughout the experiment.

Groups	*n*	Females with ovulations (%)	Ovulation rate (mean ± SEM)	Pregnancy rate[1] (%)	Fertility[2] (%)	Prolificacy (mean ± SEM)
Isolated	12	0[a]	–	–	–	–
4 h	18	94.4[b]	2.0±0.12[a]	94.4[a]	66.7[a]	1.7±0.14[a]
8 h	18	100[b]	1.7±0.14[a]	94.4[a]	61.1[a]	1.6±0.15[a]
12 h	18	100[b]	1.9±0.11[a]	88.9[a]	72.2[a]	2.1±0.14[a]
16 h	18	100[b]	1.9±0.10[a]	77.8[a]	66.7[a]	1.8±0.11[a]

[a,b]Values with different letters within each column are different ($P<0.001$).
[1] Percentage of pregnant females detected as pregnant by ultrasonography 52 days after the introduction of males.
[2] Percentage of females that kidded.

finding extends the results obtained by Rivas-Muñoz et al. (2007) which showed that daily contact of 16 h induced the same ovulatory response as full daily contact.

In addition, the percentages of does that ovulated in the first and second ovulations induced by the males in both experiments, as well as the percentages and length of the ovulatory cycles observed in experiment 1, did not differ among the four experimental groups and were similar to those reported in does exposed for 24 h to males (Flores et al., 2000; Delgadillo et al., 2002). In both experiments and in all groups, the percentages of females that displayed short ovulatory cycles were higher than the percentages of females that displayed normal ovulatory cycles, which is similar to what is observed when females are exposed continuously to sexually active males (Flores et al., 2000; Delgadillo et al., 2002). Ovulation rates were not altered by decreasing the period of contact between sexes. Indeed, they were similar in all groups exposed to males and similar to those of previous studies (Rivas-Muñoz et al., 2007; De Santiago-Miramontes et al., 2008). Taken together, these results indicate that continuous contact between anovulatory female goats and sexually active males is not necessary for a successful male effect to occur. Furthermore, our present findings show for the first time that the duration of contact between males and females can be dramatically shortened without reducing the efficacy of the stimulation by the male effect.

Several and non-exclusive explanations for the similarly high ovulatory responses of females in experiments 1 and 2 can be proposed:

First, several facts suggest that male behaviour is probably important. In goats and sheep, in all studies in which females have been exposed to olfactory cues alone, the percentage of females that ovulate reaches about 50% at most, even though olfactory cues are continuously present. Therefore, other sensory cues have to be involved, especially in the case of intermittent stimulation. The behaviour of the male appears important in this respect, as demonstrated by the recent results of Vielma et al. (2009). These authors found that males rendered sexually active by a treatment with long days as in the present experiment failed to maintain a high level of LH secretion in females for more than 24 h of exposure if the males were sedated, whereas LH secretion was maintained in females if the males—also rendered sexually active by long days—were not sedated. These results, taken together with those of the present study, indicate that olfactory cues participate in triggering and maintaining LH secretion, but that the intense sexual behaviour of males is probably a key component to reach a sufficiently high concentration of LH to lead to ovulation in the case of a short duration of contact.

A second possibility is that the daily repetition of stimulation that included both olfactory cues and high sexual behaviour of males allowed a threshold of LH and FSH levels that led to ovarian follicular development and ovulation. Indeed, it has been suggested that each re-introduction of sexually active males induces LH pulses, and that those successive occurrences of LH pulses are sufficient to stimulate follicular growth, LH peak, and ovulation (Delgadillo et al., 2009). Furthermore, the extent of the effect obtained may be enhanced by the presentation of a different male each day, which would cause a greater stimulation of the females' sexual behaviour (Pearce and Oldham, 1988; Cushwa et al., 1992); this could compensate for the diminution of the period of contact. Indeed, it is classically established in female hamsters that presenting a new partner stimulates recovery of sexual behaviour, contrary to repeating the presentation of a familiar sexual partner ("Coolidge effect"; Lisk and Baron, 1982). Similarly, in sheep, it was shown that novel males, but not familiar ones, increased LH pulse frequency in anovulatory ewes (Hawken et al., 2008). However, in goats the onset of ovulatory activity of female goats does not depend on prior contact with males, but on the intensity of sexual behaviour displayed by them (Véliz et al., 2006). In the present study we cannot conclude whether males' novelty, sexual behaviour, or the association of those are responsible for females' ovulatory response. More studies are needed to investigate the effect of daily introduction of bucks, in particular the LH secretion profile of females between and during each introduction of males.

Third, female sexual behaviour may also have influenced the response of other females to the male effect. Indeed, various studies have shown that females in estrus can stimulate other females via the female effect (Walkden-Brown et al., 1993b; Restall et al., 1995; Zarco et al., 1995; Álvarez et al., 1999). In the present study, it is possible that the first females displaying estrous behaviour and ovulatory activity as a result of the male effect may have participated in the stimulation of other does. In other words, first females presenting estrous behaviour may have played a "complementary" role by mounting other females especially in the absence of the buck.

Fourth, another possibility is that windborne delivery of male odours travelled beyond the 100 or 200 m used to separate animals in this study and was responsible for the response. Therefore, females may have been stimulated even outside the period of contact with males. Indeed, we know from the perceptual experience of human that the buck odour detected by the human nose can travel long distances. However, it has also been demonstrated that the corresponding chemical component responsible for the characteristic strong odour of bucks for humans does not possess pheromonal activity (Birch et al., 1989). Furthermore, it must be noted that when olfactory cues are provided alone to anestrous does, only about half of females ovulate even if the does have direct access to these cues (Chemineau et al., 1986; Claus et al., 1990; Walkden-Brown et al., 1993a), which is much less than the responses encountered here. Finally, several previous experiments using the same or shorter distances of isolation between sexes demonstrated that females in contact with sexually inactive males do not ovulate even when sexually active males are present in pens located 100 to 200 m away (Delgadillo et al., 2002; Rivas-Muñoz et al., 2007; Vielma et al., 2009). In those experiments, sexually active bucks induced an effective male effect only at close quarters. Therefore, it is very unlikely that possible windborne delivery of males' odour was responsible for the ovulatory response of females in the present study, even if the exact distance at which the olfactory cues cease to be perceived remains to be specified.

Finally, a last possibility that could have contributed to a high response in the females of experiment 2, would be that the olfactory cues from the males were transferred to the females' bodies during the periods of contact between males and females. Indeed, rubbing the body of the female is part of male courtship behaviour and males may have transferred relevant olfactory cues to the coat of the females through this behaviour. In this case, olfactory cues from the males may have kept stimulating females even when they remained in the "resting" pens during the daily period of separation from bucks. This residual stimulation could have then contributed to the high response observed in experiment 2 and may also explain to some extent the high response of the females regardless of the daily duration of contact between the goats and the bucks.

To conclude, results of the present study show that in anovulatory female goats, a 4 h daily contact with sexually active males is sufficient to trigger an ovulatory response providing rates of fertility that are commercially acceptable. This finding has a practical implication concerning reproductive management of goats: a given male could be used to stimulate several groups of females per day, thus multiplying the potential of males used for the male effect. For example, one sexually active male could stimulate simultaneously at least 2 groups of anovulatory females with the condition of being present 4 h a day in each group during 15 days. While our results show that discontinuous presence of males enables a high ovulatory response in goats in seasonal anestrous, more studies are needed to clarify the role played by the various cues provided by the buck in inducing the response in the female and whether females are affected by residual cues between daily exposures.

Acknowledgments

The authors are grateful to Alejandro Sandoval and Pedro Sandoval for facilitating the does used in the present study, to all members of the Centro de Investigación en Reproducción Caprina (CIRCA) of the University Antonio Narro for their technical assistance and to Dolores Lopez for her excellent secretarial and administrative support. We also express our thanks to the Assay Laboratory of the Station de Physiologie de la Reproduction et des Comportements for carrying out the radioimmunoassays.

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2 One hour of daily contact with sexually active males is sufficient to induce fertile ovulation in anestrous goats.

One hour of daily contact with sexually active males is sufficient to induce fertile ovulation in seasonal anestrous goats

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ABSTRACT

The objective of the study was to determine whether sexually active males are able to stimulate the sexual activity of anestrous female goats when duration of contact is reduced to less than 4 daily hours. One group of anovulatory females remained isolated from males (control group) and three other groups were exposed to sexually active males for 4, 2, or 1 h per day during 15 consecutive days. More than 89 % of females exposed to the sexually active bucks ovulated, whereas only 5 % did so in the control group ($P<0.001$) and this proportion did not differ among groups of females depending on duration of contact with bucks ($P=0.28$). In groups exposed to males, the ovulation rates did not differ significantly depending on the daily duration of contact with males ($P=0.13$). Furthermore, pregnancy rates, fertility and prolificacy were not affected by the daily duration of contact with males ($P=0.15$, $P=0.20$ and $P=0.86$, respectively). We conclude that one daily hour of contact with sexually active males is sufficient to stimulate fertile ovulatory activity in seasonal anovulatory goats.

Keywords: Male effect, Anovulatory does, Duration of contact, Ovulation.

INTRODUCTION

In breeds of ewes and goats that show reproductive seasonality, photoperiod is the main cue responsible for the alternation between breeding and rest seasons. Changes in photoperiod modulate the negative feedback of estrogens on the reproductive axis and the increased responsiveness to this negative feedback prevents sustained increases of LH thus provoking seasonal anestrus (Karsch *et al*., 1980; Chemineau *et al*., 1988; Maeda *et al*., 1988; Duarte *et al*., 2008). However, the introduction of a male into a group of females in seasonal anestrous can rapidly induce an increase in plasma LH levels leading to ovulation within 72 h (Cohen-Tannoudji *et al*., 1986; Martin *et al*., 1986; Claus *et al*., 1990; Delgadillo *et al*., 2003; Vielma *et al*., 2008). This biostimulation, known as the “male effect”, has been extensively studied in ewes and goats (ewes: Underwood *et al*., 1944; Signoret, 1980; Martin *et al*., 1986; goats: Shelton, 1960; Chemineau *et al*., 2006; Delgadillo *et al*., 2009). The LH secretion and ovulation of females exposed to the male effect may be influenced by factors such as the duration of contact between sexes and the intensity of the males’ sexual behavior.

Since the male effect is investigated, it has been thought that continuous presence of the male is required to maximize the endocrine and ovulatory responses of females to the male stimulus (Signoret and Lindsay, 1982; Oldham and Pearce, 1983). In both goats and ewes, the first exposure to males induces a rapid activation of LH secretion, but the stimulation of ovulation needs a longer duration of contact between sexes (Martin *et al*., 1980; Signoret *et al*., 1982;

Ungerfeld *et al*., 2004; Vielma *et al*., 2009). Indeed, it has been shown that as soon as the male or its odor (buck hair) is withdrawn, GnRH or LH secretion decreased. These results suggest that the stimulation must be continuous to maintain high gonadotropic secretion allowing ovulation (Oldham and Pearce, 1983; Ichimaru *et al*., 1999; Hawken and Martin, 2012). In fact, in sheep, only 18 % of ewes ovulated when exposed to males for 24 h, whereas 53 % ovulated when the males were maintained for 4 days and 61 % if males were maintained 15 days (Signoret *et al*., 1982). However, recent studies indicate that it is possible to decrease the duration of contact between sexes to 4 h per day and induce a high ovulatory response (> 85 %) and fertility (> 58 %) in Mexican goats (Bedos *et al*., 2010; Bedos *et al*., 2012). A likely explanation is that bucks induced to a high sexual activity during the sexual rest by a treatment of long days were used in these studies. Indeed, it was found in both goats and ewes, that a high sexual behavior of males is a key component to obtain a good ovulatory response of females to the male effect (Perkins and Fitzgerald, 1994; Flores *et al*., 2000; Delgadillo *et al*., 2002). In goats, LH pulsatility of females remained elevated at least 24 h after introduction of a light-treated awake buck which displayed an intense sexual behavior, whereas in the females in contact with the light-treated sedated buck which prevents their sexual behavior, pulsatility decreased after the first 4 h of stimulation by the buck (Vielma *et al*., 2009). In addition, the proportion of females that ovulated when exposed to light-treated awake bucks was greater than in that of females exposed to the light-treated sedate bucks (90 vs 0 %, respectively; Martínez-Alfaro *et al*., 2011).

Given the results recently obtained with sexually active bucks, the objective of this study was to determine whether sexually active males are able to stimulate the sexual activity of anestrous female goats when duration of contact is reduced to 2 or 1 daily hour during 15 days. We hypothesized that sexually active bucks would be able to stimulate the sexual activity of anestrous females when duration of contact is reduced to less than 4 h per day. To test this hypothesis, we exposed seasonally anovulatory females for 4, 2 or 1 h daily to sexually active bucks for 15 consecutive days.

MATERIAL AND METHODS

Conditions of the study

The study was carried out during the non-breeding season using local goats (Capra hircus) from the Laguna region in the State of Coahuila, Mexico (latitude, 26°23'N and longitude, 104°47'W). In females, non-breeding season lasts from March to August and in bucks from January to April (Delgadillo *et al*., 1999; Duarte *et al*., 2008). All females had given birth between August and September and were milked manually once daily during the study. Females and males were fed 2 kg of alfalfa hay (18 % CP) and 200 g of commercial concentrate (14 % CP; 1.7 Mcal/kg) with free access to water during the study.

The animals were maintained under good management conditions that fulfilled the nutritional requirements of the animals and the procedures used in the experiments were in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Males. Six bucks were kept together in an open pen (6 x 6 m). The males were subjected to a treatment of long days (16 h of light/8 h of darkness) from November 1st to January 15th. On January 16th, the light treatment was stopped and the bucks were exposed to natural variations of day-length until the end of the study (April 8th). This treatment has previously been shown to take a further 45 to 60 days to produce a stimulatory effect on male reproductive activity (Delgadillo *et al*., 2002). Testosterone secretion is stimulated from the end of February to the end of April and, as a consequence, the intensity of odor

and the sexual behavior of bucks are improved during these months corresponding normally to the non-breeding season (Delgadillo *et al*., 2002; Rivas-Muñoz *et al*., 2007). For this reason, light-treated males are used in March-April. On March 23th, bucks were individually submitted to behavioral tests in order to confirm their high sexual behavior before beginning the experiment. Every buck was exposed for 15 min to an anestrous goat and the following sexual behaviors were recorded: self urination, ano-genital sniffing, nudging, mounting attempts and mounts with vaginal intromission (Gonzalez *et al*., 1988; Fabre-Nys, 2000; Flores *et al*., 2000). From the 6 bucks rendered sexually active by the light treatment, 3 groups (n=2 each) balanced for sexual behavior were formed.

Females. Eighty-one multiparous anovulatory female goats were used. On March 18th and 23th, the ovulatory activity of females was assessed by ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe in order to determine their ovarian cyclicity. This method was previously described by Ginther and Kot (1994) and proved to be reliable for the assessment of luteal activity in goats (Simões *et al*., 2007; Delgadillo *et al*., 2011). On March 24th, anovulatory females were divided into four groups balanced for body condition score (BCS) and kept in shaded open pens (6 x 4 m): the control group (n = 20; BCS: 2.6 ± 0.1: mean ± SEM) was isolated from males. The other three groups were exposed daily to sexually active males for 4 (n = 18; 2.5 ± 0.1), 2 (n = 22; 2.5 ± 0.1), or 1 h (n = 21; 2.5 ± 0.1). BCS was assessed by palpating the spinous and lateral processes, and the musculature

of the lumbar region of the spine and allocating a score from 1 (very lean) to 4 (fat) in increments of 0.5 (Walkden-Brown *et al*., 1997).

Male effect. On March 25th (day 0), females of each experimental group were exposed to one of the 3 pairs of bucks treated with long days and males remained with females 15 days. Each group was divided into two sub-groups so that each buck stimulated individually 9 to 11 females. Bucks were introduced each day at 08:00. In groups of does exposed to males for 4, 2 and 1 h, bucks were removed from pens at 12:00, 10:00 and 09:00 h respectively, and then placed until next day in another pen located at more than 200 m from the experimental pens. In addition, the distance between the 5 groups of females was more than 100 m, thus preventing any risk of interference by the treatments between groups (Walkden-Brown *et al*., 1993).

Measurements

Females. Daily blood samples were taken from day 1 to 10 and 18 after exposure to males to assess ovarian activity by measuring the plasma concentrations of progesterone. The percentages of females ovulating before day 4 or ovulating at least once during the whole sampling were inferred from plasma concentrations of progesterone. In addition, ovulation rate was assessed by the number of corpora lutea observed in each female by transrectal ultrasonography 18 days after introduction of the bucks. Transrectal ultrasonography was performed using an Aloka SSD-500 machine connected to

a 7.5 MHz linear probe. Pregnancy rate (pregnant does/does exposed to males) was determined by abdominal ultrasonography 56 days after exposure to males using the same machine connected to a 3.5 MHz abdominal probe. Fertility (number of females kidding/number of females exposed to males) and prolificacy (number of kids born/number of females giving birth) were determined at parturition.

Progesterone assay. Concentrations of plasma progesterone were measured by RIA as described by Saumande *et al*. (1985). Sensitivity was 0.25 ng/mL. The intra- and inter-assay coefficients of variation were 7 and 3 % respectively. Females with progesterone concentrations ≥ 0.5 ng/mL were considered to have ovulated (Chemineau *et al*., 2006).

Statistical analyses

The Chi-square test was used for multiple-group comparisons and the Fisher's exact probability test was used for two-group comparisons to assess statistical differences in the proportions of females that ovulated before day 4 or at least once during the study, pregnancy rates and fertility. Ovulation rate and prolificacy were all compared using the Kruskall-Wallis-test followed by the Mann-Whitney U test. Analyses were computed using SYSTAT (2009) with significance set at $p<0.05$ and expressed as the mean ± standard error of the mean.

RESULTS

Response of females to the male effect: ovulatory activity, fertility and prolificacy

More than 89 % of females exposed to the sexually active bucks ovulated at least once during the whole experiment, whereas only 5 % did so in the control group ($P<0.001$; Table 1). Moreover, this proportion was similar among groups of females exposed for 1, 2, or 4 h per day to sexually active males ($P=0.28$; Table 1). However, the proportion of goats ovulating before day 4 was higher in the 2- or 4-hour contact groups than in the control one ($P=0.02$ and $P<0.001$, respectively) whereas it was similar between the control group and the group of females in contact for 1 daily hour with males ($P=0.23$; Table 1). In groups exposed to males, the ovulation rates did not differ significantly depending on the daily duration of contact with males ($P=0.13$). Finally, pregnancy rates, fertility and prolificacy were similar among groups of females exposed to males ($P=0.15$, $P=0.20$ and $P=0.86$, respectively; Table 1).

Table 1. Short- (day 1-4) and long-term (day 1-18) ovulatory and reproductive responses of anestrous female goats exposed for 1, 2 or 4 h to males rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th.

Groups	n	Short-term ovulatory response	Long-term ovulatory response		Reproductive response		
		Females ovulating between day 1–4 (%)	Females ovulating between day 1-18 (%)	Ovulation rate (Mean ± SEM)	Pregnancy rate[1] (%)	Fertility[2] (%)	Prolificacy (Mean ± SEM)
Isolated	20	0[a]	5[a]	-	-	-	-
1 h	21	14[ab]	100[b]	2.0 ± 0.15[a]	71[a]	62[a]	1.8 ± 0.15[a]
2 h	22	27[bc]	95[b]	1.6 ± 0.13[a]	91[a]	77[a]	1.8 ± 0.13[a]
4 h	18	50[c]	89[b]	1.8 ± 0.10[a]	67[a]	50[a]	1.8 ± 0.10[a]

[1] Percentage of females detected pregnant by ultrasonography 56 days after the introduction of males
[2] Percentage of females that kidded
[a,b,c] Different superscripts within each column denote significant difference ($P<0.05$).

DISCUSSION

Our results show that sexually active males are able to stimulate the sexual activity of anovulatory females even when the daily contact between them is reduced to one hour. Interestingly, the ovulatory and reproductive variables that we measured did not differ among groups exposed to males for 4, 2 or 1 h per day at the end of the 15 days of study. Moreover, our data are similar to previous studies in which females were exposed for 24, 16, 12, 8 or 4 h per day to sexually active males (Bedos *et al*., 2010; Bedos *et al*., 2012; Flores *et al*., 2000). However, we observed a delay in the short-term ovulatory response. Indeed, only 19 % of females in contact 1 h per day with males ovulated before day 4, whereas half of them did so in the 4-hour contact group. Nonetheless, after 15 days of contact between sexes, those differences were canceled and the proportions of females ovulating were high and did not differ between groups of females in contact with males, independently of the duration of contact. As a whole, those findings validated our hypothesis that sexually active bucks are able to stimulate anestrous females being in contact with them 1 h per day but the initial ovulatory response is delayed compared to longer durations of contact.

An explanation of the delay of the initial ovulatory response could be that the intermittent presence of males failed to maintain a permanent high LH secretion, thus delaying ovulation. Indeed, a previous study showed that, when males are removed from a group of ewes, LH secretion decreases to its initial level 2 h after males' withdrawal (Oldham and Pearce, 1983). Nonetheless in the

current study, we can suppose that each re-introduction of the male induced an increase in LH pulse frequency, which enabled to stimulate the ovulatory activity of the females, since high percentages of goats ovulated after 15 days of contact with bucks (Delgadillo *et al*., 2009). Overall, the response observed in the present study could be due to the fact that long-day treated bucks were used in the present study. Indeed, only photoperiodic-treated bucks, compared to control ones, are able to stimulate the sexual activity of females during the seasonal anestrous (Flores *et al*., 2000; Delgadillo *et al*., 2002). The efficiency of the photoperiodic-treated bucks relies on the fact that they display a range of powerful cues such as an intense sexual behavior, a strong odor and high frequency vocalizations that stimulate estrous behavior and ovulation in females (Flores *et al*., 2000; Delgadillo *et al*., 2002; Rivas-Muñoz *et al*., 2007; Delgadillo *et al*., 2012). Specifically, they recently demonstrated that light-treated males' sexual behavior and vocalizations are fundamental cues that are responsible for the high LH secretion and ovulatory response of females (Delgadillo *et al*., 2012). Indeed, sedate light-treated bucks failed to induce ovulation in anestrous goats comparing to awake ones (Martínez-Alfaro *et al*., 2011) and in goats that have had previous contact with males, the sound of males' vocalizations alone increased LH secretion and induced ovulation in 33 % of them (Delgadillo *et al*., 2012). Finally, other recent studies using a 4-hour daily contact between sexes also pointed out the importance of male sexual activity to obtain a high ovulatory response of females (Bedos *et al*., 2010; Bedos *et al*., 2012). Taken together, our results and those mentioned above indicate that the intense sexual behavior

and all the sensorial cues that emanate from light-treated males must have been crucial factors in our study.

In conclusion, the present study shows for the first time that one daily hour of contact between anovulatory goats and long-day treated males during 15 days is sufficient to stimulate the females' sexual activity very efficiently. These results suggest that the male effect could be used as an even more powerful technique of biostimulation during the seasonal anestrus.

ACKNOWLEDGEMENTS

The authors are grateful to Alejandro Sandoval for facilitating the does used in the present study, to all members of the Centro de Investigación en Reproducción Caprina (CIRCA) of the Universidad Autónoma Agraria Antonio Narro for their technical assistance, and to Dolores Lopez for her excellent secretarial and administrative support. We also would like to acknowledge the laboratory of hormonal determinations of the Station de Physiologie de la Reproduction et des Comportements of the INRA of Nouzilly, France, for carrying out the progesterone assays. Marie Bedos was supported by a scholarship of Secretaría de Relaciones Exteriores during her doctoral studies. This work was supported by grants from the SEP-PROMEP (Red "Conducta sexual y reproductiva en caprinos y ovinos"), the "Programa de Cooperación de Posgrado" between Mexico and France (03/09: "El efecto macho en cabras: tiempo de contacto entre los sexos y mecanismos

subyacentes) and the “Programa de Cooperación CONACYT (México) - ANR (France) (159884: “El efecto macho en ungulados: reproducción aplicada y mecanismos neuroendocrinos) / ANR Blanche internationale France-Mexique (11-ISV7-001-01).

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3 Sexually active bucks are able to stimulate three successive groups of females per day with a 4-hour period of contact

Physiology & Behavior 106 (2012) 259–263

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

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ARTICLE INFO

Article history:
Received 24 November 2011
Received in revised form 24 January 2012
Accepted 12 February 2012
Available online 18 February 2012

Keywords:
Male effect
Anovulatory does
Duration of contact
Male–female ratio
Ovulation

ABSTRACT

Bucks rendered sexually active by a photoperiod treatment of long days can induce fertile ovulation in a group of goats with only 4 h of contact daily with a male:female ratio of 1:10. Here we tested whether such bucks could induce fertile ovulations when stimulating successively three different groups of anovulatory goats when interacting 4 h per day during 15 consecutive days. Control males ($n=3$) were introduced in the control group ($n=25$) of does at 8:00 h and were removed at 12:00 h. Experimental males ($n=3$) were in contact with the experimental groups of does: from 8:00 h to 12:00 h with a first group ($n=27$), from 12:00 h to 16:00 h with a second group ($n=26$) and with a third one ($n=27$) from 16:00 h to 20:00 h. Bucks were then placed until next day in another pen. Both in the control and the experimental groups, more than 85% of females ovulated, and the proportions did not differ between the control and experimental groups ($P\geq 0.67$) or between the three experimental groups ($P\geq 0.67$). Moreover, the ovulation rate did not differ significantly between the control and the experimental females nor between the three experimental groups. Bucks were able to fertilize more than 72% of does independently of the number of females they were exposed to ($P\geq 0.17$). Finally, more than 58% of females kidded and fertility did not differ between the control and experimental groups ($P=1$) nor among experimental groups ($P\geq 0.77$). We conclude that sexually active bucks are able to induce fertile ovulation in three successive groups of anovulatory goats even when the period of contact between sexes is reduced to 4 h per day.

1. Introduction

In breeds of sheep and goats that show a period of seasonal anestrous, the introduction of a male into a group of anovulatory females can stimulate their sexual activity within 72 h. This phenomenon, known as the "male effect", has been extensively studied in ewes [1–3] and goats [4–6]. Factors such as the duration of contact between sexes, the intensity of the males' sexual behavior and the male:female ratio may influence the response of females exposed to males [6].

In ewes, it has been shown that the presence of the male 24 h per day during 15 days is required to obtain a maximum ovulatory response [7]. However, recent studies indicate that the decrease of the duration of contact between sexes from 24 to 16, 12, 8 or 4 h per day still enables to induce a high ovulatory response and fertility in Mexican goats [8,9]. A likely explanation for the difference between the studies in ewes [7] and goats [8,9] is that long-days-treated – and therefore sexually active – bucks were used in these two latter studies, whereas in the first one, they used rams that had not been treated with light and which were therefore probably either in sexual rest or with a low sexual behavior. Indeed, it was found in both goats and ewes, that a high sexual activity of males is a key component to obtain a high ovulatory response of females to the male effect [10,11].

A recent study in goats indicates that the females' estrous response is not affected by decreasing male:female ratio but that some specific variables are [12]. Indeed, Carrillo et al. [12] found that a decrease in the male:female ratio from 4:39 to 2:39 or 1:39 had no effect on the occurrence of estrous behavior but lengthened mean interval between introduction of males and onset of estrous behavior. In our breeding conditions, several experiments have shown that a male:female ratio from 1:8 to 1:13 [10,13–15] ensures proper fertilization of females and, more recently, it was demonstrated that such a ratio is sufficient to induce high ovulatory and reproductive responses in females even when daily contact between the buck and the females is reduced to 4 h [9]. However, whether sexually active bucks are able to induce ovulation, sexual activity and adequate fertilization to three groups of anestrous goats stimulated successively when the daily period of contact is 4 h remains to be investigated. Considering that a male:female ratio of 1:10 enable high ovulatory and reproductive responses,

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doi:10.1016/j.physbeh.2012.02.015

it could be expected that sexually active bucks will be able to stimulate three groups of females when the ratio is in this range and the daily period of contact is 4 h. To test this possibility, we exposed daily sexually active bucks either to a single or to three different groups of females during 4 h, at a ratio of 1:9 and for a period of 15 consecutive days.

2. Materials and methods

2.1. Conditions of the study and females

The experiment was performed using local goats (*Capra hircus*) from the Laguna region in the State of Coahuila, Mexico (latitude, 26°23′N and longitude, 104°47′W). In females, non-breeding season lasts from March to August, and in bucks from January to April [16,17]. All females were multiparous and had given birth between October and December and were milked manually once daily during the study. Females and males were fed 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate (14% CP; 1.7 Mcal/kg) with free access to water during the study.

All the females used were anovulatory at the beginning of the experiment. To ensure this, on March 15th and 23th, each female was submitted to a transrectal ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe in order to determine their ovarian cyclicity. This method was previously described by Ginther and Kot [18] and proved to be reliable for the assessment of luteal activity in goats [19]. On March 24th, anovulatory females were divided into four groups balanced for live weight and body condition score and kept in open pens (6×4 m; Table 1). Body condition score was assessed by palpating the spinous and lateral processes, and the musculature of the lumbar region of the spine and allocating a score from 1 (very lean) to 4 (fat) in increments of 0.5 [20].

2.2. Sexual activation of bucks – photoperiodic treatment, semen quality and sexual behavior

Six bucks were kept together in an open pen (6×6 m). The males were subjected to a treatment of long days (16 h of light/8 h of darkness) from November 1st to January 15th. On January 16th, the light treatment was stopped and the bucks were exposed to natural variations of day-length until the end of the study. This treatment has previously been shown to take a further 45 to 60 days to produce a stimulatory effect on male reproductive activity [10]. Testosterone secretion is stimulated from the end of February to the end of April and, as a consequence, the production of male odor and the sexual behavior of bucks are improved during these months corresponding normally to the non-breeding season [8,10]. For this reason, light-treated males are used in March–April. On March 17th, the quality of semen was assessed in undiluted ejaculates by determining the progressive sperm motility and the percentage of live spermatozoa observed immediately after semen collection [21]. Semen was collected using an artificial vagina, when males were presented to an intact estrus-induced doe. All males had good quality semen with more than 70% of live spermatozoa and sperm motility greater than three (0: no movements; 5: rapid and rectilinear movements [21]). On March 22nd, bucks were individually submitted to behavioral tests in order to confirm their high sexual behavior before beginning the experiment. Every buck was exposed to an anestrous goat and the following sexual behaviors were recorded: self urination, ano-genital sniffing, nudging, mounting attempts and mounts with vaginal intromission [22,23]. From the 6 bucks rendered sexually active by the light treatment, two groups ($n=3$ each) balanced for semen quality and sexual behavior were formed: a group of control males and a group of experimental males.

Table 1
Characteristics of the goats of the control group (C) that were exposed to three males from 8:00 h to 12:00 h and the experimental groups that were exposed to three other males from 8:00 h to 12:00 h (G8–12), from 12:00 h to 16:00 h (G12–16) and from 16:00 h to 20:00 h (G16–20). Each group of females was in contact with males 4 h per day during 15 days. Males were rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th.

Groups	*n*	Live weight kg (Mean ± SEM)	BCS[a] (Mean ± SEM)
C	25	42 ± 1	1.9 ± 0.1
G8–12	27	41 ± 1	1.9 ± 0.1
G12–16	26	41 ± 1	1.9 ± 0.1
G16–20	27	41 ± 1	1.9 ± 0.1

[a] BCS = body condition score (range 1 to 4).

2.3. Male effect

On March 27th (day 0), females were exposed to bucks for 15 days. Control males were in contact with one group of females from 8:00 h to 12:00 h (C). Experimental males were in contact with three successive groups of females: with the first group from 8:00 h to 12:00 h (G8–12); with the second group from 12:00 h to 16:00 h (G12–16) and with the third group from 16:00 h to 20:00 h (G16–20). Each group was divided into three sub-groups so that each buck stimulated individually 8 or 9 females. After the daily period of contact, bucks were placed together until the next day in another pen located at more than 200 m from the females' pens. In addition, the distance between the four groups of females was more than 100 m, thus preventing any risk of interference by the treatments between groups or sub-groups [24]. Females remained in the pens of stimulation during the whole experiment. The experimental protocol is showed in Fig. 1.

2.4. Measurements

2.4.1. Females

The male-induced ovulation and ovulation rate were assessed by the presence and number of corpora lutea, respectively, observed in each female by transrectal ultrasonography with the same equipment as above, 20 days after introduction of the bucks [18,19]. Pregnancy rate (pregnant does/does exposed to males) was determined by abdominal ultrasonography 60 days after exposure to males using the same machine connected to a 3.5 MHz abdominal probe. Fertility (number of females kidding/number of females exposed to males) and prolificacy (number of kids born/number of females giving birth) were determined at parturition.

2.4.2. Males

Sexual behavior of the bucks was observed on days 1, 2, 7 and 8 after their introduction into the groups of females. Each day, the observations were carried out during the first hour of contact between sexes in each group of females i.e. from 8:00 h to 9:00 h in C and G8–12; from 12:00 h to 13:00 h in G12–16 and from 16:00 h to 17:00 h in G16–20. Trained observers followed bucks individually and recorded the following behaviors: self urination, ano-genital sniffing, nudging, mounting attempts and mounts with vaginal intromission [22,23].

2.5. Statistical analyses

The Chi-square test was used for multiple-group comparisons and the Fisher's exact probability test was used for two-group comparisons to assess statistical differences in the proportions of females that ovulated, pregnancy rates and fertility. Ovulation rate and prolificacy were all compared using the Kruskall–Wallis-test followed by the Mann–Whitney *U* test. Males' behavioral components were analyzed with a Chi-square test for goodness of fit considering a random distribution of 50% in each group when comparing control and experimental

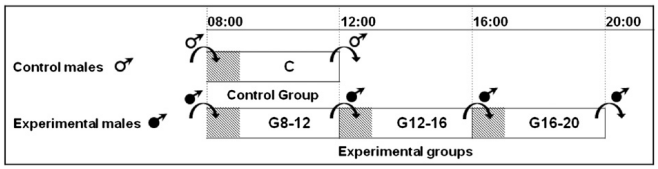

Fig. 1. Experimental design showing movements (curved arrows) of bucks rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th within groups of females. Three control males (open male symbol) daily stayed from 8:00 h to 12:00 h with one group of control females (C). Three experimental males (filled male symbol) daily stayed with three different groups of experimental females: from 8:00 h to 12:00 h in G8–12, from 12:00 h to 16:00 h in G12–16 and from 16:00 h to 20:00 h in G16–20. Those were the experimental groups. Hatched zones indicate times when males' sexual behavior was observed on day 1, 2, 7 and 8 after their introduction.

males from 8:00 h to 9:00 h. The Chi-square test for goodness of fit was also used when comparing experimental males' behavioral components at different times of observation (from 8:00 h to 9:00 h, from 12:00 h to 13:00 h and from 16:00 h to 17:00 h, respectively), considering a random distribution of 33.3% at each time of observation. Analyses were computed using SYSTAT 10 [25] with significance set at $P<0.05$ and expressed as the mean ± standard error of the mean.

2.6. Ethical note

The animals were maintained under good management conditions that fulfilled the nutritional requirements of the animals and the procedures used in the experiments were in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching [26].

3. Results

3.1. Response of females to the male effect: ovulatory activity, fertility and prolificacy

More than 85% of females ovulated during the whole experiment, independently of the group of bucks they were exposed to. Indeed, the proportion of goats that ovulated did not differ between control females and experimental ones ($P\geq0.67$) or between the three groups of experimental females ($P\geq0.67$; Table 2). The ovulation and pregnancy rates did not differ significantly between control females and experimental ones ($P=0.62$ and $P\geq0.17$, respectively) or between the three groups of experimental females ($P=0.42$ and $P\geq0.18$, respectively; Table 2). More than 58% of females kidded and fertility did not differ between control females and experimental ones ($P=1$) or between the three groups of experimental females ($P\geq0.77$, Table 2). Finally, prolificacy did not differ between control females and experimental ones ($P=0.27$) or between the three groups of experimental females ($P=0.15$, Table 2).

Table 2
Ovulatory response, pregnancy rate, fertility and prolificacy of anestrous goats daily exposed to three males from 8:00 h to 12:00 h (C) or exposed to three other males from 8:00 h to 12:00 h (G8–12), from 12:00 h to 16:00 h (G12–16) or from 16:00 h to 20:00 h (G16–20). Each group of females was in contact with males 4 h per day during 15 days. Males were rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th.

Groups	Females with ovulations (%)	Ovulations rate (Mean ± SEM)	Pregnancy rate[a] (%)	Fertility[b] (%)	Proficacy (Mean ± SEM)
C	92	1.8 ± 0.14	72	64	1.7 ± 0.15
G8–12	85	1.8 ± 0.12	81	63	1.6 ± 0.15
G12–16	92	2.0 ± 0.12	76	58	1.9 ± 0.12
G16–20	93	1.8 ± 0.10	89	63	1.8 ± 0.11

[a] Percentage of females detected pregnant by ultrasonography 60 days after the introduction of males.
[b] Percentage of females that kidded.

3.2. Sexual behavior of bucks

Experimental males displayed more mounting attempts than control ones from 8:00 h to 9:00 h ($P<0.001$; Fig. 2). Besides, the occurrence of various sexual behaviors in experimental males was significantly different depending on the time of observation. Indeed, experimental males displayed more nudging and mounting attempts from 8:00 h to 9:00 h than from 12:00 h to 13:00 h or 16:00 h to 17:00 h ($P<0.001$), but there was no difference between 12:00 h to 13:00 h and 16:00 h to 17:00 h ($P\geq0.86$; Fig. 3). Finally, experimental males displayed more mountings with intromission from 8:00 h to 9:00 h than from 12:00 h to 13:00 h ($P=0.038$) but there was no difference between the periods 8:00 h to 9:00 h and 16:00 h to 17:00 h or 12:00 h to 13:00 h and 16:00 h to 17:00 h ($P\geq0.18$; Fig. 3).

4. Discussion

Our results show that sexually active males are able to stimulate the ovarian activity of three groups of anovulatory goats during a 4-hour daily interaction, as efficiently as when in contact with only one group. Indeed, in the present study, the proportion of females ovulating was high in all experimental groups and, more interestingly, was similar to that of the control group. Likewise, globally, bucks were able to fertilize more than 72% of does and pregnancy rates did not differ between experimental groups and the control group. In addition, other reproductive variables such as ovulation rate and prolificacy did not differ between experimental groups and the control group and they were similar to those reported in previous studies in which duration of contact was reduced to 16 h or 4 h per day [8,9]. As a whole, our results are in accordance with previous studies which used continuous (24 h per day) contact [13] or a 4-hour contact per day between sexes [9], both with a 1:10 male:female ratio. Those findings validate our

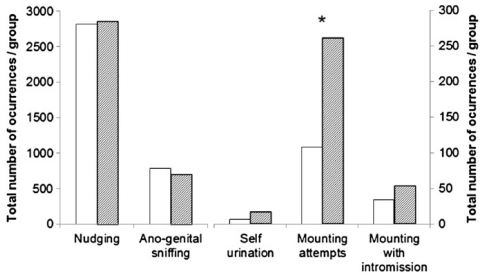

Fig. 2. Frequencies of nudging, ano-genital sniffing, self urination, mounting attempts and mountings with intromission of control males (open bars) and experimental males (hatched bars) rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th observed from 8:00 h to 9:00 h on day 1, 2, 7 and 8 after buck introduction. * Denotes significance within one behavior characteristic ($P=0.05$).

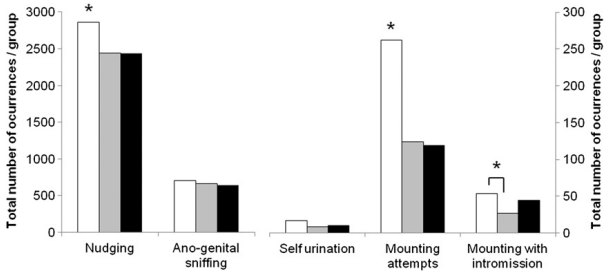

Fig. 3. Frequencies of nudging, ano-genital sniffing, self urination, mounting attempts and mountings with intromission in experimental males rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th observed at 8:00 h (open bars), 12:00 h (shaded bars) and 16:00 h (solid bars) during 1 h on day 1, 2, 7 and 8 after buck introduction *Denotes significance within one behavior characteristic ($P = 0.05$).

hypothesis that sexually active bucks are able to induce fertile ovulation in various groups of anovulatory females being in contact with them 4 h per day during 15 consecutive days.

Our results could be explained by the following arguments. Firstly, in our study, males were submitted to a photoperiodic treatment that was demonstrated to stimulate males' sexual behavior during the non-breeding season. Our results strongly suggest that the intense males' sexual behavior is a key element for the females' response and various studies are in accordance with this argument. Indeed, Flores et al. [13] showed that 100% of female goats displayed estrus when they were exposed to bucks rendered sexually active by exposing them to artificial long days followed by melatonin treatment. Similar results were demonstrated by Rivas-Muñoz et al. [8] and Delgadillo et al. [10] when they used bucks exposed to artificial long days followed by natural photoperiod. In contrast, when non light-treated males, and therefore sexually inactive are used, the proportion of females ovulating is very low [10,13]. Taken together, our results and those mentioned above indicate that the intense males' behavior is a crucial factor to obtain a high response in females and we can suppose that it probably participated in the ovulatory response we observed in the experimental groups in our study. Secondly, the male:female ratio used in our experiment may also explain the females' response we obtained. Indeed, in our study, the male:female ratio was 1:9 in each group. This ratio is similar to those used in previous experiments that employed long-days-treated bucks in contact 24 h [13] or 4 h per day [9] with females which displayed high estrous and ovulatory responses. Similar results were reported with long-days-treated bucks in continuous contact with females using a ratio male:female that varied from 4:39 to 1:39 [12]. In our study, even if the ratio was 1:9 in each sub-group, the total number of goats stimulated by one experimental male reached 27 per day. This number of females per male is in accordance with programs of breeding management of goat reproduction in conditions of natural mating that recommend a ratio of one buck for 25 to 30 females. It would be of utmost interest to investigate how much the male:female ratio can be further decreased with the same experimental design of our study or add a fourth group o females. As a whole, we can conclude that thanks to the high male:female ratio that was maintained in each sub-group (1:9), the experimental bucks were able to stimulate three consecutive groups of females with a 4-hour period of contact.

Bucks that were in contact with the experimental groups of females displayed more mounting attempts than bucks in contact with the control group of goats. A likely explanation for this result is that the contact of experimental males with a greatest number of females per day may have enhanced their level of mount attempts. Therefore, the increased number of mount attempts could be a consequence of the greater interaction the experimental males had with females. Bucks which stimulated three groups of females performed more nudging, mounting attempts and mounting with intromission in the morning than at midday or in the afternoon. An explanation for this phenomenon is that the bucks could rest at night. Indeed, experimental males were taken away from females for 12 h so we can suppose that they were more rested during the morning period of observation than in the consecutive periods at midday and in the afternoon. Another possibility is that the environmental conditions at 8:00 h were more favorable for males' sexual behavior than at 12:00 h or at 16:00 h. Indeed, studies carried out in the same region as our experiment found that, in March and April, outdoor temperature varies from 8 ºC in the morning to 32 ºC in the afternoon [17,27]. This possibility is supported by the results of Lindsay [28] who observed that when temperature is gradually raised from 26 ºC to 43 ºC, reproductive activity of rams starts to be inhibited at 32 ºC. Nonetheless, even though the sexual behavior of experimental males diminished between the morning and midday or afternoon, the ovulatory response and pregnancy rates of experimental does were similar in all groups. Taken together, our results showed that sexual behavior displayed by the experimental bucks was sufficient to stimulate the reproductive activity of goats even if they suffered a diminution of their sexual activity throughout the day.

In conclusion, results of the present study show that sexually active males are able to trigger very good ovulatory and reproductive responses in anovulatory goats even when the duration of contact between sexes is reduced and the number of groups of females is increased to three per day. Moreover, from a practical standpoint, our results suggest that sexually active males could be used more efficiently than they used to be, by reducing the duration of contact and increasing the number of groups of females stimulated by day.

Acknowledgements

The authors are grateful to Alejandro Sandoval for facilitating the does used in the present study, to all members of the Centro de Investigación en Reproducción Caprina (CIRCA) of the Universidad Autónoma Agraria Antonio Narro for their technical assistance, and to Dolores Lopez for her excellent secretarial and administrative support. We also thank Pascal Poindron for his comments on the manuscript. Marie Bedos was supported by a scholarship of Secretaría de Relaciones Exteriores during her doctoral studies. This work was supported by grants from the SEP-PROMEP (Red "Conducta sexual y reproductiva en caprinos y ovinos"), the "Programa de Cooperación de Posgrado" between Mexico and France (03/09: "El efecto macho en cabras: tiempo de contacto entre los sexos y mecanismos subyacentes) and the "Programa de Cooperación CONACYT" (México) – ANR (France) (159884: "El efecto macho en ungulados: reproducción aplicada y mecanismos neuroendocrinos)/ANR Blanche internationale France-Mexique" (11-ISV7-001-01).

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DISCUSIÓN GENERAL

Los resultados de los 3 estudios que conforman la presente tesis indican que el contacto de 24 h por día, entre cabras anovulatorias locales del subtrópico de México y machos cabríos foto-estimulados, no es necesario para estimular las actividades ovulatoria y reproduciva en la mayoría de las hembras. En efecto, estos resultados demuestran que el tiempo de contacto entre hembras y machos puede ser drásticamente reducido a 1 h por día sin disminuir la eficacia del estímulo proporcionado por los machos foto-estimulados. En los dos primeros estudios, los porcentajes de hembras expuestas a machos foto-estimulados que ovularon, así como las tasas ovulatorias, no fueron diferentes estadísticamente al reducir el tiempo de contacto a 16, 12, 8, 4, 2 y 1 h por día. Asimismo, los porcentajes de hembras gestantes, la fertilidad y prolificidad observados en estos dos estudios, no difirieron según la duración de contacto entre los sexos. En conjunto, los resultados obtenidos al reducir el tiempo de contacto de 16 a 1 h por día, son similares a los reportados en estudios en los cuales las hembras estuvieron en contacto 24 h por día con machos foto-estimulados (Flores *et al*., 2000; Delgadillo *et al*., 2002 Rivas-Muñoz *et al*., 2007; De Santiago-Miramontes *et al*., 2008). En el tercer estudio se demostró que los machos cabríos foto-estimulados son capaces de estimular las actividades ovulatoria y reproductiva de 3 grupos diferentes de hembras anovulatorias interractuando con ellas 4 h diarias. En efecto, los machos experimentales, que estuvieron en contacto con 3 grupos diferentes de hembras

diariamente, fueron capaces de inducir la ovulación y fertilizar una proporción de hembras similar a la inducida por los machos control, que estuvieron en contacto 4 h por día solamente con un grupo de hembras. Los resultados del tercer estudio son similares a los reportados en aquellos en los cuales las hembras estuvieron en contacto 24 h por día con machos foto-estimulados (Flores *et al*., 2000; Delgadillo *et al*., 2002; Delgadillo y Vélez, 2010). Los resultados de los 3 estudios que conforman esta tesis, demuestran por primera vez en la especie caprina, que la eficacia del efecto macho se puede incrementar al reducir el tiempo de contacto entre los dos sexos a 1 h por día, y/o estimular a 3 grupos diferentes de cabras con los mismo macho foto-estimulados al ponerlos en contacto 4 h por día con cada uno de estos grupos.

Los resultados obtenidos en los 3 estudios que conforman esta tesis, pueden deberse al hecho que los machos cabríos fueron foto-estimulados al someterlos a 2.5 meses de DL a partir del 1 de noviembre. En efecto, los machos foto-estimulados, a diferencia de los machos control, en reposo sexual, son capaces de estimular la actividad sexual de las hembras durante el anestro estacional (Flores *et al*., 2000; Delgadillo *et al*., 2002). La eficacia de los machos foto-estimulados puede deberse al hecho de que despliegan un comportamiento sexual intenso, un olor fuerte y una frecuencia alta de vocalizaciones, señales que estimulan las actividades endocrina, estral y ovulatoria de las hembras anéstricas (Flores *et al*., 2000; Delgadillo *et al*., 2002; Vielma *et al*., 2009; Martínez-Alfaro *et al*., 2011; Delgadillo *et al*., 2012). Al respecto, recientemente se demostró que el comportamiento sexual y las

vocalizaciones de los machos foto-estimulados son las señales fundamentales para estimular la secreción de LH y la ovulación de las hembras anéstricas (Delgadillo *et al*., 2012; Martínez-Alfaro *et al*., 2011). Por ello, los machos foto-estimulados sedados, que no despliegan comportamiento sexual, no inducen la ovulación en cabras anéstricas; en cambio, los machos foto-estimulados despiertos, que despliegan un intenso comportamiento sexual, inducen la ovulación en la mayoría de las hembras (90%; Martínez-Alfaro *et al*., 2011). Asimismo, las vocalizaciones "in vivo" de los machos foto-estimulados incrementan la secreción de LH de hembras anéstricas e inducen la ovulación en el 33% de ellas (Delgadillo *et al*., 2012). Los resultados de la presente tesis sugieren que el comportamiento sexual intenso y las vocalizaciones de los machos foto-estimulados, son factores cruciales para las respuestas ovulatoria y reproductiva de las hembras expuestas a los machos cabríos foto-estimulados.

En el segundo estudio, las primeras ovulaciones inducidas por el macho se retrasaron en las cabras expuestas 1 h por día a los machos, en relación con aquellas que permanecieron en contacto con éstos 4 h por día. Una explicación a este fenómeno podría ser que el perfil de secreción de LH sea diferente entre las cabras expuestas a los machos por 1 o 4 h al día. En efecto, cuando un carnero se introdujo en un grupo de ovejas anéstricas se incrementó la secreción de LH. Sin embargo, cuando el macho se retiró, la secreción de LH disminuyó 2 h después de haber retirado el macho, al nivel basal registrado antes del contacto entre los dos sexos (Oldham y Pearce, 1983). En el presente estudio podemos suponer que la secreción de LH disminuyó en la ausencia de

los machos, pero que cada re-introducción de éstos estimuló la frecuencia de los pulsos de LH. Si la presencia del macho estimula la secreción de LH, los niveles plasmáticos de esta hormona deben permanecer elevados menos tiempo en las cabras expuestas a los machos 1 h, que en aquellas expuestas a 4 h. Este perfil diferente en la secreción de LH pudo retrasar la primera ovulación en las cabras que permanecieron en contacto con los machos 1 h por día (Delgadillo *et al*., 2009).

Los resultados que conforman esta tesis, y otros reportados previamente, demuestran que los machos deben estar foto-estimulados para ser capaces de inducir las actividades ovulatoria y reproductiva de las cabras durante el anestro estacional (Flores *et al*., 2000; Delgadillo *et al*., 2002). La capacidad de los machos foto-estimulados para inducir las actividades sexual y reproductiva de las cabras anéstricas puede explicarse por las siguientes hipótesis:

1) Es probable que los machos foto-estimulados modifiquen el efecto inhibitorio de los estrógenos sobre el eje gonadotrópico. En efecto, en las ovejas ovariectomizadas que portan un implante subcutáneo de estradiol (OVX+E), la introducción de un carnero estimula la secreción de LH. Sin embargo, este incremento en la secreción de LH es menor en las hembras OVX, lo que llevó a los autores a la conclusión de que la presencia del macho modifica la retroalimentación negativa del estradiol sobre el eje gonadotrópico (Martin *et al*., 1983). En nuestras condiciones experimentales, los machos foto-estimulados que despliegan un intenso comportamiento sexual estimulan la secreción de LH y la ovulación a pesar de la alta sensibilidad del eje

hipotalamo-hipofisiario a la retroalimentación negativa de los estrógenos (Karsch *et al*., 1980; Mori *et al*., 1987; Chemineau *et al*., 1988; Vielma *et al*., 2009; Martínez-Alfaro *et al*., 2011). En cambio, los machos foto-estimulados sedados que no despliegan un comportamiento sexual, no estimulan la secreción de LH ni la ovulación (Vielma *et al*., 2009; Martínez-Alfaro *et al*., 2011). Estos resultados sugieren que en los 3 estudios de la presente tesis, el comportamiento sexual de los machos foto-estimulados es un factor importante para inducir la actividad ovulatoria y reproductiva de las cabras en anestro estacional debido a la probable modificación de la retroalimentación negativa del estradiol sobre el eje gonadotrópico.

2) Es probable que las señales exteroceptivas provenientes de los machos foto-estimulados activen las neuronas que secretan kisspeptina (Hawken y Martin, 2012). La kisspeptina es un neuropéptido que juega un papel fundamental en la regulación de la secreción de GnRH, y en consecuencia de la LH (Smith *et al*., 2006; Oakley *et al*., 2009). En efecto, en ovejas y cabras, la aplicación exógena de kisspeptina estimula la secreción de LH y la ovulación (Messager *et al*. 2005; Caraty *et al*. 2007; Ohkura *et al*., 2009; Hashizume *et al*., 2010). Estos resultados demuestran que la kisspeptina controla, muy probablemente, la secreción del GnRH, y en consecuencia, de la LH y la ovulación. Además, se demostró en cabras que el olor a macho provoca una activación de las células a kisspeptina y que el aumento de la actividad eléctrica de estas células está asociado a los pulsos de LH (Murata *et al*., 2011). En ratones, la exposición de las hembras a la orina del macho activa las neuronas a kisspeptina (Bakker *et al*., 2010). Estos resultados demuestran que en cabras y ratones, las señales

olfativas provenientes de los machos estimulan la secreción de la kisspeptina. Es muy probable que la presencia del macho estimule directamente las células a kisspeptina pasando por alto la retroalimentación negativa que ejercen los estrógenos sobre estas neuronas (Smith *et al*., 2007; 2008). En nuestras condiciones experimentales, podemos suponer que la presencia de los machos foto-estimulados activaron las neuronas que secretan la kisspeptina, que a su vez estimuló la secreción de GnRH y LH, lo que permitió que ocurriera la ovulación en las cabras anéstricas.

CONCLUSIONES

Los resultados de los 3 estudios de la presente tesis demuestran por primera vez en la especie carina, que la reducción del tiempo de contacto entre machos y hembras no disminuye la respuesta ovulatoria y reproductiva de las cabras expuestas al efecto macho durante el anestro estacional. En los estudios 1 y 2, se demostró que la duración de contacto puede ser reducida de 16 a 1 h diaria sin disminuir la respuesta ovulatoria y reproductiva de las cabras expuestas al efecto macho. En el estudio 3 se demostró que los machos foto-estimulados pueden inducir las actividades ovulatoria y reproductiva de 3 grupos de hembras con un contacto diario de 4 h con cada grupo. En conjunto, estos resultados demuestran que los machos cabríos foto-estimulados pueden ser utilizados de manera más eficiente al reducir el tiempo de contacto diario con las hembras y aumentar el número de grupos estimulados por un macho. En ambos casos se aumenta considerablemente el número de hembras estimuladas por macho.

PERSPECTIVAS

Los resultados de la presente tesis abren nuevas perspectivas de investigación en cuanto a los mecanismos neuroendocrinos involucrados en las respuestas ovulatoria y reproductiva observadas en los presentes estudios. Sería interesante determinar si existen diferencias en los patrones de secreción de LH en cabras expuestas a machos foto-estimulados por 24 o 2 h por día, y si éstos diferentes patrones de secreción inducen a la ovulación. Asimismo, sería interesante investigar si la presencia de los machos foto-estimulados modifican la retroalimentación negativa del estradiol sobre la LH. Finalmente, sería interesante determinar si la presencia de machos foto-estimulados estimula la secreción de kisspeptina, que a su vez estimularía el eje hipotálamo-hipófisis-gónadas. El estudio de los mecanismos neuroendocrinos involucrados en la estimulación de las cabras anéstricas a través del efecto macho, contribuirá a la comprensión del funcionamiento de esta técnica sustentable de bio-estimulación sexual.

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