La reducción en la duración de los tratamientos fotoperiódicos de los machos cabríos, y del tiempo de contacto entre machos y hembras, no disminuyen la eficiencia del efecto macho.

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“Ninguna fuerza doma, ningún tiempo consume, ningún mérito iguala, ***el nombre de la libertad****” Nicolás Maquiavelo, 1469-1527.*

COMPENDIO

La reducción en la duración de los tratamientos fotoperiódicos de los machos cabríos, y del tiempo de contacto entre machos y hembras, no disminuyen la eficiencia del efecto macho.

Por

JOSÉ LUIS PONCE COVARRUBIAS

DOCTOR EN CIENCIAS AGRARIAS

UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

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En los machos cabríos está documentado que los tratamientos fotoperiódicos que incluyen la exposición de 75 días largos (DL) alternados con el fotoperiodo natural correspondiente a días cortos (DC) crecientes, son eficientes para inducir una intensa actividad sexual durante el periodo de reposo sexual natural. Además, cuando estos machos foto-estimulados son puestos en contacto con hembras anovulatorias durante al menos 15 días estimulan su actividad estral y ovulatoria.

Sin embargo, no se conoce si los tratamientos de DL menores a 75 días reducen la respuesta sexual de los machos a dicho tratamiento, así como su capacidad para estimular la actividad ovulatoria de las hembras anéstricas durante el efecto macho. Asimismo, no se conoce si un tiempo de contacto macho-hembra menor a 15 días reduce la respuesta ovulatoria. Por ello, en la presente tesis se llevaron a cabo 3 experimentos para aclarar los planteamientos anteriores.

En el estudio 1 se realizaron dos experimentos para determinar i) si la disminución de los tratamientos de DL a 45, 30, o 15 días, no reduce la respuesta sexual de los machos cabríos al tratamiento; ii) determinar si los machos foto-estimulados con 45 o 30 DL inducen la actividad ovulatoria en las cabras anéstricas. En ambos experimentos se utilizaron hembras y machos cabríos locales de la Comarca Lagunera, ubicada en el subtrópico mexicano (26°N). En el experimento 1 se usaron 25 machos cabríos adultos. Un grupo de machos (n=5) se expuso a las variaciones naturales del fotoperiodo, mientras que 4 grupos (n=5/grupo) se expusieron a 16 horas de luz por día durante 75, 45, 30, o 15 DL artificiales. En los 4 grupos los tratamientos fotoperiódicos terminaron el 15 de enero. Las concentraciones plasmáticas de testosterona se determinaron una vez por semana del 15 de octubre al 30 de mayo. El aumento de los niveles plasmáticos de testosterona ocurrió primero en los machos de los grupos de 75 y 45 DL artificiales que en los machos de los grupos de 30, 15 DL, y el testigo ($P<0.05$). Además, el tiempo durante el cual los niveles plasmáticos de testosterona fueron elevados (>5 ng/mL), fue mayor en los machos de 75 y 45 DL artificiales que en los grupos de 30 y 15 DL artificiales ($P<0.05$). En el experimento

2, un grupo de cabras anovulatorias (n=13) se aisló de los machos, mientras que 3 grupos adicionales (n=25, 27, y 26 hembras/grupo) se pusieron en contacto durante 15 días con machos previamente expuestos a 75, 45, o 30 DL artificiales (n=3 machos por grupo). La proporción de cabras que ovularon fue mayor en los 3 grupos en contacto con los machos foto-estimulados (88%, 89% y 92%, respectivamente) que en el grupo aislado (0%; $P<0.05$). La proporción de cabras preñadas no difirió ($P>0.05$) entre los 3 grupos en contacto con los machos foto-estimulados (78%, 85% y 92%; respectivamente). En conclusión, la reducción del tratamiento fotoperiódico hasta 15 días de DL artificiales durante el otoño-invierno, estimula la secreción de testosterona de los machos cabríos durante el periodo de reposo sexual. Además, los machos foto-estimulados con 45 y 30 DL son capaces de inducir la ovulación en las cabras durante el anestro estacional.

El estudio 2 se realizó para determinar si la disminución del tiempo de contacto entre machos y hembras no reduce la actividad ovulatoria de las cabras cuando son expuestas a los machos foto-estimulados por 1, 5, o 10 días. En este experimento se utilizaron hembras y machos cabríos locales de la Comarca Lagunera, ubicada en el subtrópico mexicano (26°N). Cuatro machos se sometieron a un tratamiento de 75 DL artificiales (16 horas luz/día) a partir del 1 de noviembre, seguido del fotoperiodo natural, para estimular su actividad sexual durante el periodo de reposo. El grupo testigo de hembras (n=12) fue expuesto durante 15 días a un macho foto-estimulado. Otros tres grupos de hembras (n=14 o 15 por grupo) fueron expuestas a los machos (n=1 macho por grupo) por 1, 5, o 10 días. Las ovulaciones se determinaron por las concentraciones plasmáticas de progesterona en muestras sanguíneas obtenidas diariamente durante los primeros 16 días post-introducción de los machos. Todas las hembras de los grupos testigo y experimentales ovularon al menos una vez durante todo el experimento (P>0.05). Las proporciones de cabras que mostraron ciclos ovulatorios cortos (47%, 67%, 71% y 67%) o normales (13%, 20%, 29% y 33%) no difirieron entre los grupos dependiendo de la duración del tiempo de contacto con los machos foto-estimulados (P>0.05). En contraste, la proporción de hembras que desarrolló un cuerpo lúteo de corta vida y luego retornó al anestro fue mayor en las cabras que estuvieron en contacto con los machos por 1 día (33%), que en aquellas que estuvieron en contacto con los machos por 10 o 15 días (0% en ambos grupos; P<0.05). Ninguna diferencia existió de esta variable entre las hembras expuestas a los machos por 1 o 5 días (20%; P>0.05). En conclusión, 1, 5, o 10 días de contacto con machos foto-estimulados es suficiente para estimular la actividad ovulatoria en cabras anéstricas sometidas al efecto macho.

Palabras clave: Estacionalidad reproductiva, testosterona; comportamiento sexual, efecto macho, duración del contacto, ovulación.

ABSTRACT

In male goats is documented that the photoperiodic treatments that include the exposure to 75 long days (LD) alternating with short days (DC) corresponding to increasing natural photoperiod, are efficient to induce an intense sexual activity during the natural sexual rest. Furthermore, when these males photo-stimulated are contacted with anovulatory females for at least 15 days, stimulate estrus and ovulatory activity. However, it isn´t known whether treatments of less than 75 days DL reduce male sexual response to treatment, as well as its ability to stimulate ovulatory activity of anestrus females during the male effect. Also isn´t known whether time male-female contact less than 15 days reduces the ovulatory response. Therefore, in this thesis were conducted three experiments to clarify the above statements.

In the study 1, two experiments were conducted to determine i) the decrease in DL treatments at 45, 30, or 15 days, does not reduce the sexual response of male goats to treatment; ii) determine whether photo-stimulated males 45 or 30 DL induce ovulation in anovulatory goats activity. In both experiments females and males local goats from the Comarca Lagunera, located in the Mexican subtropics (26°N) were used. In experiment 1 was used 25 adult goats. A group of males (n=5) was exposed to the photoperiod natural variations, while 4 groups (n=5/group) were exposed to 16 hours of light per day for 75, 45, 30, or 15 DL artificial. In the 4 groups, photoperiodic treatments ended on January 15th. Plasma concentrations of testosterone were determined in blood samples obtained once a week from October 15th to May 30th. The rise of testosterone levels occurred earlier in males from the 75-LD and 45-LD groups than in those from the 30-LD, 15-LD, and control groups (*P*<0.05). In addition, the time during which levels of testosterone remained high >5 ng/mL was longer in males from the 75-LD and 45-LD than in those from the 30-LD and 15-LD groups (*P*<0.05). In experiment 2, a group of anovulatory goats (n=13) was isolated from males, while 3 additional groups (n=25, 27, and 26 females/group). Were put in contact during 15 days with males previously exposed to 75, 45, or 30 days of LD. The proportion of goats that had ovulated was higher in the 3 groups in contact with the photo-stimulated males (88%, 89% y 92%, respectively) than in the group isolated from them (0%; *P*<0.05). The proportion of pregnant females did not differ (*P*>0.05) between the 3 groups of does in contact with photo-stimulated males (78%, 85% y 92%; *P*>0.05). We concluded that photoperiodic treatment reduced up to 15 days of artificial DL during the autumn-winter, stimulates testosterone secretion goats during the period of sexual rest. Also, these males are able to induce ovulations in seasonal anestrous goats.

Study 2 was conducted to determine whether the decrease in contact time between males and females didn´t reduce ovulatory activity of goats when exposed to photo-stimulated males for 1, 5, or 10 days. In this experiment were used females and males local goats from the Comarca Lagunera, located in the Mexican subtropics (26°N). Four males underwent treatment artificial DL 75 (16 h light/day) from November 1st, followed by natural photoperiod, to stimulate their sexual activity during the rest period. The control group of females (n=12) was exposed to one photo-stimulated buck for 15 days. Other three experimental groups of females (n=14 or 15 per group) were exposed to males (n=1 male per group) for 1, 5, or 10 consecutive days. Ovulations were determined by plasma progesterone levels in blood samples taken daily for the first 16 days post-introduction of the bucks. All females from the control and experimental groups ovulated at least once during the whole experiment ($P>0.05$). The proportions of goats in short ovulatory cycles (47%, 67%, 71% and 67%) or normal (13%, 20%, 29% and 33%) ovulatory cycles did not differ between groups depending on the duration of time of contact with the photo-stimulated males ($P>0.05$). In contrast, the proportion of females that entered in anovulation after developed a corpus luteum of short life-span was greater in the goats in contact with males for 1 day (33%), than in those in contact with males for 10 and 15 days (0% in both groups; $P<0.05$). No difference existed in this variable between females exposed to males for 1 or 5 days (20%; $P>0.05$). We concluded 1, 5, or 10 days of contact with males photo-stimulated are long enough to stimulate the ovulatory activity in seasonally anovulatory goats subject to male effect.

Keywords: Reproductive seasonality, testosterone; sexual behavior, male effect, duration of contact, ovulation.

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I. INTRODUCCIÓN

En México, la producción caprina se ha considerado a través del tiempo como una práctica ganadera asociada a productores de bajos recursos. Actualmente nuestro país cuenta con 8,743,949 cabezas de ganado, y los estados con mayor producción son Puebla, Oaxaca y Coahuila. La región de la Comarca Lagunera cuenta con 419,117 cabezas de este ganado (SIAP, 2012), y se localiza en el subtrópico Mexicano (26°N). Los caprinos locales de la Comarca Lagunera presentan estacionalidad en su actividad reproductiva, y el fotoperiodo es la principal señal del medio ambiente responsable de dicha estacionalidad (Delgadillo *et al.,* 1999, Duarte *et al.,* 2008). Las variaciones del fotoperiodo modulan la retroalimentación negativa de la testosterona en los machos y del estradiol en las hembras sobre el eje gonadotrópico, mecanismo neuroendocrino responsable de que se manifiesten periodos de actividad y reposo sexual (Chemineau *et al.,* 1988a; Karsch *et al.,* 1980; Pelletier y Ortavant, 1975). La estacionalidad reproductiva limita la disponibilidad de leche y cabrito en cierto periodo del año (Chemineau *et al.,* 2007; Delgadillo, 2011). Para enfrentar esta limitante de la producción caprina, es necesario conocer la biología del comportamiento reproductivo de los caprinos y así, tener las bases para hacer más eficiente el manejo del hato.

En los machos cabríos se puede inducir una intensa actividad sexual durante el periodo de reposo usando tratamientos fotoperiódicos que consisten en la alternancia de 75 DL artificiales a partir del 1 de noviembre seguidos de DC naturales (Bedos *et al.,* 2010; Delgadillo *et al.,* 2002; Vielma *et al.,* 2009).

Sin embargo, no se conoce la respuesta sexual de los machos cuando son foto-estimulados con 45, 30, o 15 DL artificiales. En las hembras caprinas tampoco se conoce la respuesta ovulatoria cuando son expuestas a machos que recibieron un tratamiento fotoperiódico de 45 o 30 días. En las cabras se puede usar la bioestimulación sexual conocida como "efecto macho" para inducir la actividad sexual y ovulatoria durante el anestro estacional (Delgadillo *et al.,* 2002, 2009; Flores *et al.,* 2000; Shelton, 1960). Aunque existen algunos factores que limitan la efectividad del efecto macho, incluyendo la intensidad del comportamiento sexual del macho y el tiempo de contacto entre machos y hembras (Perkins y Fitzgerald, 1994; Signoret *et al.,* 1982). Así, en cabras y ovejas, los machos que despliegan un intenso comportamiento sexual son más eficientes para estimular la actividad ovulatoria que los machos que muestran débil comportamiento sexual (Delgadillo *et al.,* 2001; Flores *et al.,* 2000; Perkins y Fitzgerald, 1994). Referente al tiempo de contacto, en ovejas se observó que la proporción de hembras que ovuló se incrementó al aumentar los días de contacto entre ambos sexos (1 día, 18%; 4 días, 53% y 13 días, 61%; Signoret *et al.,* 1982). En cambio, en cabras no se conoce la respuesta ovulatoria cuando se exponen a los machos foto-estimulados por 1, 5, o 10 días consecutivos.

Considerando los argumentos anteriores, en el presente estudio se determinará i) si los tratamientos de DL artificiales de 45, 30, o 15 días estimulan la secreción de testosterona en el periodo de reposo sexual; ii) si los machos cabríos sometidos a tratamientos de DL artificiales de 45 o 30 días son capaces de estimular la actividad ovulatoria de las cabras en anestro estacional a través del efecto macho; iii) si el tiempo de contacto por 1, 5, o 10 días entre machos y hembras, estimulan la respuesta ovulatoria de las cabras sometidas al efecto macho.

II. REVISIÓN DE LITERATURA

1 La estacionalidad reproductiva como mecanismo de adaptación al medio ambiente

En las diferentes especies de mamíferos originarias de latitudes templadas, la estacionalidad reproductiva permite que los partos se concentren en el momento del año en que el recién nacido tiene mayores posibilidades de sobrevivir (Bronson, 1989; Karsch *et al.,* 1984; Lehman *et al.,* 1997; Malpaux *et al.,* 2001). Por ello, en estas especies, la estación sexual ocurre según la duración de la gestación. En la yegua, la gestación dura alrededor de 340 días, y la actividad sexual se presenta en los meses de primavera y verano, por lo que los partos ocurren en primavera y verano del siguiente año (Allen y Wilsher, 2009; Panchal *et al.,* 1995; Silver, 1990). En la cabra y oveja, la gestación dura alrededor de 150 días, en consecuencia, su actividad reproductiva inicia en otoño-invierno para que los nacimientos ocurran en primavera (Amoah *et al.,* 1996; Anderson *et al.,* 1981; Ortavant *et al.,* 1985; Shelton, 1960).

En latitudes subtropicales, los partos de las especies de mamíferos que presentan estacionalidad reproductiva, no ocurren siempre en el mejor momento del año para favorecer la sobrevivencia de las crías. En efecto, en los caprinos y ovinos locales de latitudes subtropicales, cuando las hembras y los machos permanecen juntos durante todo el año, los partos ocurren en otoño e invierno, cuando existe una baja disponibilidad de alimento y la temperatura ambiental también es baja, por lo que ponen en riesgo la sobrevivencia de la progenie (Bedos *et al.,* 2010; Delgadillo *et al.,* 1998; Flores *et al.,* 2000; Molina *et al.,* 1997).

1.1 La estacionalidad reproductiva en los caprinos y ovinos

1.1.1 Hembras

Las razas de cabras y ovejas originarias y/o adaptadas a latitudes templadas (> 40° Norte o Sur) y subtropicales (> 23º < 40º) muestran una marcada estacionalidad reproductiva (Amoah *et al.,* 1996; Delgadillo, 2011; Malpaux *et al.,* 1996). En las cabras Alpinas y ovejas de las razas Ile de France y Suffolk, originarias de latitudes templadas mantenidas en el hemisferio norte, el periodo de actividad sexual (estro y ovulación) se presenta de septiembre-octubre a enero-febrero (otoño-invierno), y el anestro se observa de marzo a agosto (anestro y anovulación, primavera-verano; Chanvallon *et al.,* 2011; Chemineau *et al.,* 1992a,b; Karsch *et al.,* 1984). En la parte subtropical de Argentina (30° Sur), la actividad reproductiva de las cabras locales se presenta de febrero a septiembre (otoño-invierno), y la época de anestro estacional ocurre de octubre a enero (primavera-verano; Rivera *et al.,* 2003). En las cabras del subtrópico mexicano (latitud de 26° Norte), en las cuales la estación sexual se presenta de septiembre a febrero (otoño-invierno) y la época anovulatoria se observa de marzo a julio (primavera-verano; Duarte *et al.,* 2008; Figura 1).

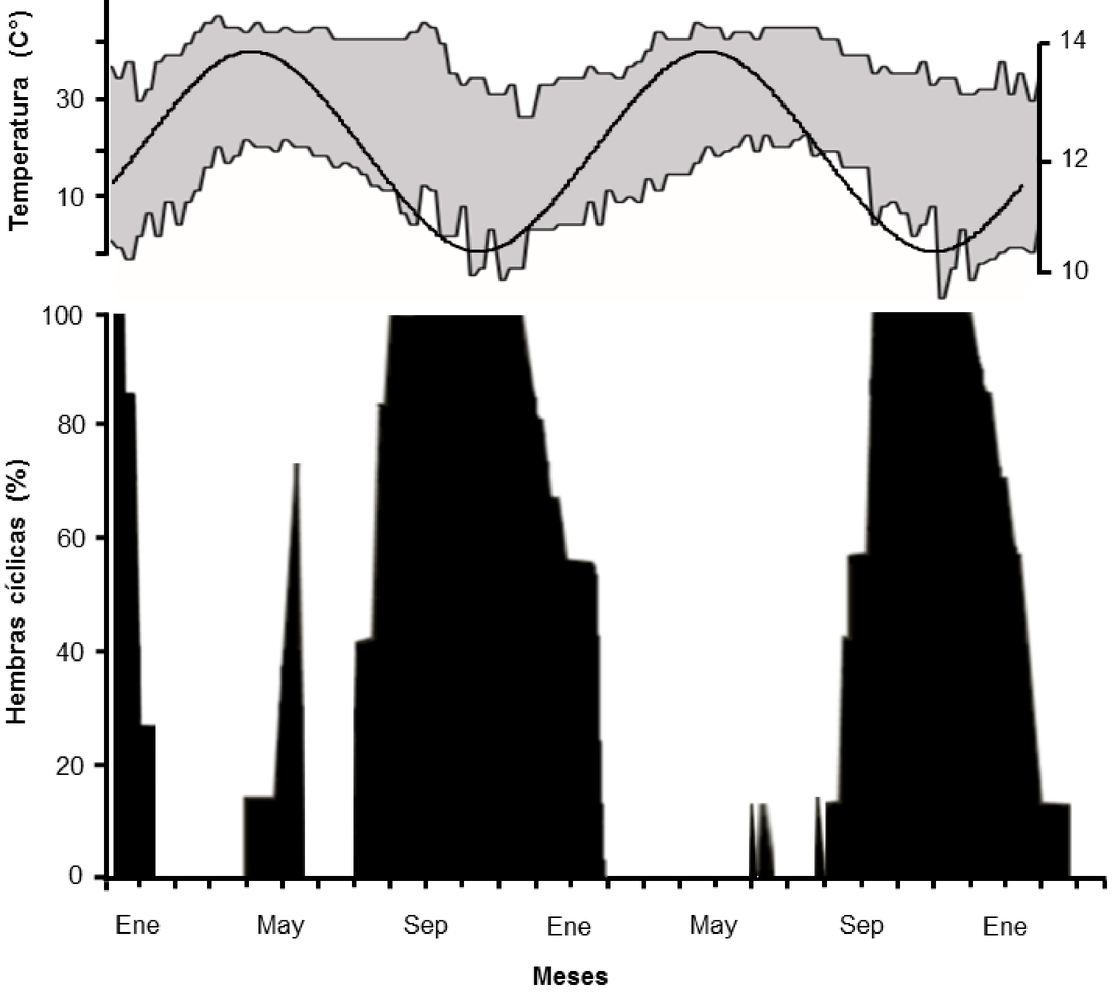

Figura 1. Variaciones estacionales de la actividad ovulatoria de las cabras locales de la Comarca Lagunera (26ºN; parte inferior) que percibieron los cambios naturales del fotoperiodo (—) y la temperatura ambiental (□, parte superior; Delgadillo *et al.,* 2004; Duarte *et al.,* 2008).

1.1.2 Ciclo estral de las cabras y ovejas

Las cabras y ovejas que presentan estacionalidad reproductiva son clasificadas como poliéstricas estacionales, es decir, presentan varios ciclos estrales y ovulatorios durante la estación sexual. El ciclo estral es el tiempo transcurrido entre dos celos consecutivos. El ciclo ovulatorio es el tiempo transcurrido entre dos ovulaciones consecutivas. Generalmente los celos son asociados con las ovulaciones. En los ciclos estrales las hembras presentan modificaciones hormonales, anatómicas y de comportamiento (Bartlewski *et al.,* 2011; Chemineau *et al.,* 1992b; Fatet *et al.,* 2011).

El ciclo estral en la cabra tiene una duración promedio de 21 días, y se divide en dos fases: folicular y lútea (Driancourt, 2001; Jarosz *et al.,* 1971; Medan *et al.,* 2003; Rosa y Bryant, 2003). La fase folicular se subdivide en dos etapas, proestro y estro. El proestro tiene una duración promedio de 3 días, e inicia a partir de la regresión del cuerpo lúteo; este se caracteriza por una disminución en la secreción de progesterona, y por un rápido crecimiento folicular e incremento en la secreción de estradiol por los folículos (Bartlewski *et al.,* 2011; Medan *et al.,* 2003; Phillips *et al.,* 1943). Durante el proestro, llamado también periodo de "dominancia folicular", la FSH secretada por la adenohipófisis estimula el crecimiento folicular favoreciendo la selección de 2 o 3 folículos con diámetro > 4 mm que continúan su crecimiento hasta la ovulación. El resto de los folículos degeneran, debido al fenómeno de "atresia folicular" (Driancourt, 2001; Fatet *et al.,* 2011; Medan *et al.,* 2003). El estro o celo es el periodo de receptividad sexual y tiene una duración promedio de 3 días, la cual puede variar según la raza, edad, estación del año, y presencia o ausencia del macho (Camp *et al.,* 1983; Fatet *et al.,* 2011; Rosa y Bryant, 2003; Shelton, 1978). Las concentraciones altas de estradiol secretado por los folículos, son las responsables de la aparición del estro. Además, el estradiol actúa por retroalimentación positiva sobre el eje hipotálamo-hipófisis incrementando la secreción de la hormona liberadora de gonadotropinas (GnRH), que a su vez induce el pico pre-ovulatorio de LH, el cual provoca la ovulación de 30 a 36 horas después del inicio del estro (Akusu *et al.,* 1986; Bartlewski *et al.,* 2011).

La fase lútea también se subdivide en metaestro y diestro (Chemineau *et al.,* 1992a; Fatet *et al.,* 2011). El metaestro tiene una duración promedio de 3 días, aunque puede variar de 2 a 5 días, y es la etapa siguiente a la ovulación, durante la cual disminuye la secreción de estradiol. En este momento, las células de la granulosa y teca interna del folículo que ovuló, inician su “luteinización” o formación del cuerpo lúteo bajo la influencia de la LH (Fatet *et al.,* 2011). El diestro tiene una duración promedio de 12 días, y se caracteriza por la presencia del cuerpo lúteo funcional, el cual secreta progesterona (Jarosz *et al.,* 1971; Medan *et al.,* 2003). La progesterona actúa por retroalimentación negativa sobre las gonadotropinas, LH y FSH, por lo que las concentraciones plasmáticas son bajas durante el diestro. En caso de que ocurra la fecundación, el cuerpo lúteo persiste manteniendo elevadas las concentraciones plasmáticas de progesterona y por lo tanto la gestación. En caso contrario, el cuerpo lúteo es destruido por acción de la prostaglandina F2α secretada por el útero, provocando la disminución de las concentraciones plasmáticas de progesterona, y el fin de la retroalimentación negativa sobre el eje hipotálamo-hipófisis, permitiendo el inicio de un nuevo ciclo estral (Driancourt, 2001; Fatet *et al.,* 2011; Figura 2).

En las cabras, la duración promedio del ciclo estral es de 21 días (rango: 17 a 25 días). Sin embargo, existen ciclos estrales y ovulatorios de corta duración (< 17 días) y larga duración (> 25 días; Jarosz *et al.,* 1971; Camp *et al.,* 1983; Chemineau, 1983; Chemineau *et al.,* 1992a; Cerbito *et al.,* 1995). En las cabras y ovejas, los ciclos estrales de corta duración son más frecuentes al inicio de la pubertad o de la estación sexual, al final del anestro postparto, así como cuando se utiliza el efecto macho para inducir el estro y la ovulación en el anestro estacional o lactacional (Chemineau*,* 1983; Flores *et al.,* 2000; Hunter, 1991; Poindron *et al.,* 1980). En cambio, en cabras y ovejas, los ciclos largos son más frecuentes al final de la estación sexual (Camp *et al.,* 1983; Chemineau *et al.,* 1992a; Cerbito *et al.,* 1995; Flores *et al.,* 2000). Además, en cabras y ovejas puede existir disociación entre el estro y la ovulación, es decir, se pueden presentar celos sin que ocurra la ovulación y también ovulaciones sin manifestación de celos. En las cabras, los estros sin ovulación se presentan al inicio de la pubertad o estación sexual, así como al final del anestro postparto o durante el efecto macho (Camp *et al.,* 1983; Flores *et al.,* 2000). En cambio, las ovulaciones que no son acompañadas de estro se observan al final de la estación sexual (Chemineau *et al.,* 1992a; Rivera *et al.,* 2003).

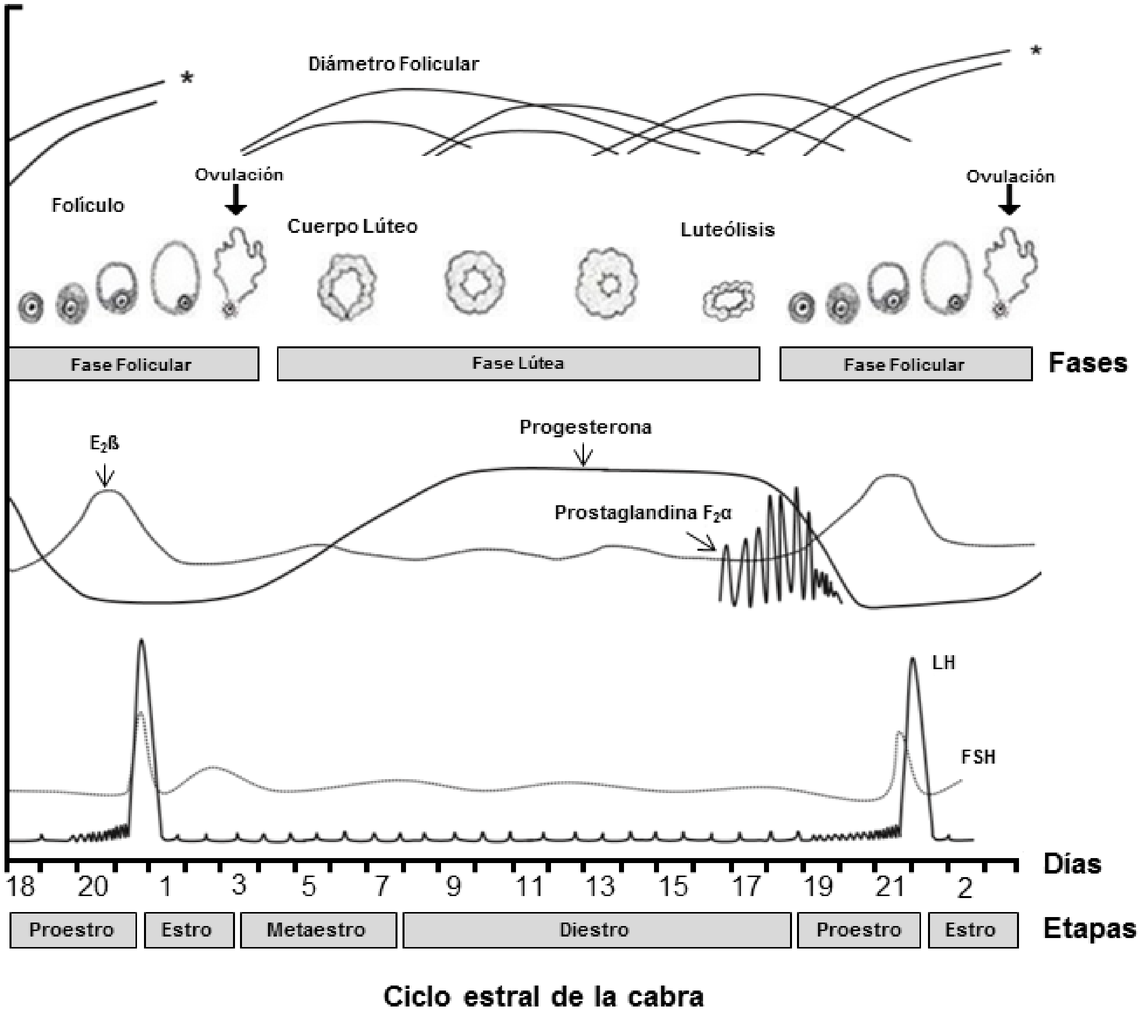

Figura 2. Representación esquemática de los eventos fisiológicos que ocurren durante el ciclo estral de la cabra; parte superior: ciclo ovulatorio y diámetro folicular; parte inferior: cambios hormonales (Fatet *et al.*, 2011).

1.2 Estacionalidad reproductiva en los machos cabríos

Los machos cabríos de latitudes templadas y subtropicales manifiestan variaciones estacionales en su actividad reproductiva. En los machos de las razas Alpina y Saanen, originarios de latitudes templadas, la estación sexual se presenta de septiembre a febrero (otoño-invierno), mientras que el periodo de reposo sexual se extiende de marzo a agosto (primavera-verano; Delgadillo *et al.,* 1991, 1992). En los machos cabríos de las razas Alpina y Saanen, el peso testicular y la producción espermática cualitativa y cuantitativamente son más elevados durante la estación sexual que en el periodo de reposo (Delgadillo *et al.,* 1991, 1992). Además, la secreción de testosterona se incrementa al inicio de la estación sexual (septiembre), y disminuye progresivamente hasta alcanzar niveles basales al final de la estación sexual (febrero; Delgadillo y Chemineau, 1992). El incremento de la secreción de testosterona mejora el comportamiento sexual de los machos, ya que se observa una disminución en la latencia a la eyaculación (Delgadillo *et al.,* 1991).

En los machos cabríos de latitudes subtropicales, la estación sexual ocurre en una época del año diferente a la que se presenta en los machos de latitudes templadas (Delgadillo *et al.,* 1999; Walkden-Brown *et al.,* 1994). En efecto, en los machos locales de la Comarca Lagunera, la estación sexual se presenta de mayo a diciembre (primavera-otoño), mientras que el periodo de reposo sexual se extiende de enero a mayo (invierno-primavera, Delgadillo *et al.,* 1999; Figura 3). En los machos cabríos locales de la Comarca Lagunera, el peso testicular y la producción espermática cualitativa y cuantitativamente son más elevados durante la estación sexual que en el periodo de reposo (Delgadillo *et al.,* 1999, 2001; Rivas-Muñoz *et al.,* 2007). Además, la secreción de testosterona se incrementa al inicio de la estación sexual (mayo), se mantiene elevada hasta noviembre y disminuye progresivamente hasta alcanzar niveles basales al final de la estación sexual (diciembre). De esta manera, durante la estación sexual, el comportamiento sexual y el olor de los machos son más intensos que durante la temporada de reposo (Delgadillo *et al.,* 1999, 2001; Flores *et al.,* 2000; Walkden-Brown *et al.,* 1994, 1997).

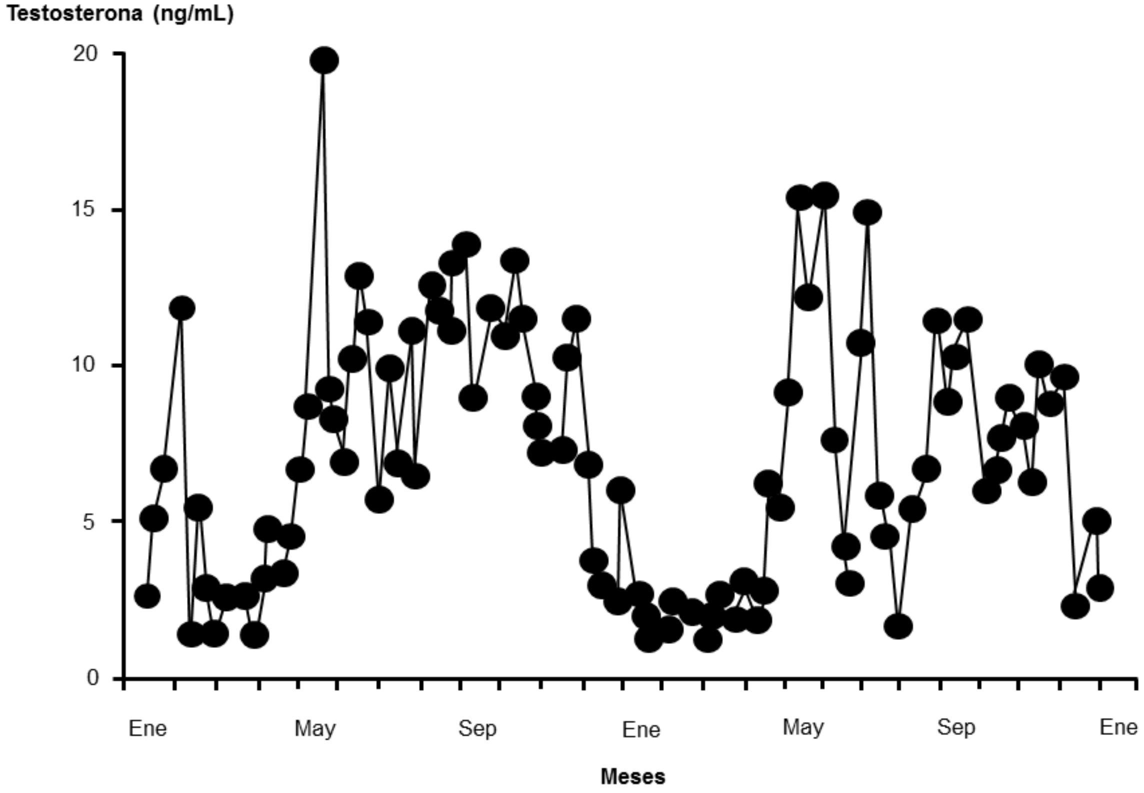

Figura 3. Variaciones anuales de las concentraciones plasmáticas de testosterona de los machos cabríos locales de la Comarca Lagunera (26°N) que percibieron los cambios naturales del fotoperiodo y la temperatura ambiental (Delgadillo *et al.,* 1999).

2 El fotoperiodo, responsable de la estacionalidad reproductiva en los caprinos y ovinos

El fotoperiodo es el principal factor del medio ambiente que sincroniza la actividad sexual anual de los caprinos y ovinos originarios o adaptados a zonas templadas o subtropicales (Chemineau *et al.,* 1992b; Earl *et al.,* 1985; Malpaux *et al.,* 1993; Martin *et al.,* 1999; Thiéry *et al.,* 2002). El efecto del fotoperiodo se demostró al someter animales a diferentes tratamientos fotoperiódicos. En efecto, en los machos y hembras caprinos y ovinos que fueron sometidos a alternancias entre 2, 3 o 4 meses de DC alternados con 2, 3 o 4 meses de DL, la actividad sexual determinada por las ovulaciones y la secreción de testosterona inició durante los DC y terminó durante los DL (Bittman *et al.,* 1983; Delgadillo *et al.,* 1991, 2004; Delgadillo y Chemineau, 1992; Duarte *et al.,* 2010; Legan y Karsch, 1983; Pelletier y Almeida, 1987). Las variaciones de la sensibilidad del eje hipotálamo-hipófisis a la retroalimentación negativa de la testosterona en los machos, así como del estradiol en las hembras, es el mecanismo neuroendocrino responsable de los periodos de actividad y reposo sexual estacional en los caprinos y ovinos (Chemineau *et al.,* 1988a; Duarte *et al.,* 2008; Karsch *et al.,* 1980, 1984; Pelletier y Ortavant, 1975). En los machos y hembras, la sensibilidad a la retroalimentación negativa a la testosterona o al estradiol aumenta durante los DL, lo que provoca una disminución en la secreción de Kisspeptina, GnRH y LH, provocando en ambos sexos periodos de reposo sexual (Boukhliq *et al.,* 1999; Clarke *et al.,* 2009; Karsch *et al.,* 1980; Kitahashi y Parhar, 2013; Pelletier y Ortavant, 1975; Smith, 2012).

Sin embargo, el efecto del fotoperiodo no es permanente. Así, cuando los animales son expuestos continuamente a DC o DL artificiales durante varios años, la actividad sexual sigue presentando variaciones, pero estas variaciones no coinciden con las estaciones del año y están desincronizadas entre los individuos (Howles *et al.,* 1982; Karsch *et al.,* 1989; Radford, 1961). Esto sugiere que la estacionalidad reproductiva en los caprinos y ovinos se debe a la existencia de un ritmo endógeno de la reproducción, que es sincronizado por el fotoperiodo (Gebbie *et al.,* 1999; Howles *et al.,* 1982; Karsch *et al.,* 1989). 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.,* 1988; Malpaux y Karsch, 1990). La existencia del ritmo endógeno de la 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.,* 1988b, 1992b).

La glándula pineal está implicada en el control del fotoperiodo sobre la reproducción. En efecto, esta glándula es la encargada de traducir la información fotoperiódica a través de la secreción de melatonina (Arendt, 1998; Karsch *et al.,* 1984; Robinson y Karsch, 1988). La información fotoperiódica es percibida por los fotorreceptores de la retina del ojo, y de ahí se transmite a los núcleos supraquiasmáticos y paraventriculares del hipotálamo. Posteriormente, la vía nerviosa desciende a la médula espinal y alcanza el ganglio cervical superior, de donde una vía postganglionar simpática inerva el pinealocito en la glándula pineal (Arendt, 1998; Legan y Karsch, 1983; Figura 4). Esta glándula secreta melatonina con un perfil día–noche bien definido. La melatonina se sintetiza y secreta solamente durante la noche, y la duración de la secreción está determinada por la duración de la noche o periodo de obscuridad. Durante los DC, cuando la secreción de melatonina es más larga, ésta es secretada por la glándula pineal directamente al líquido céfalo-raquídeo y alcanza el núcleo premamilar del hipotálamo medio basal. Posteriormente, a través de redes neuronales desconocidas, la melatonina estimula la secreción de kisspeptina por neuronas que se encuentran en el núcleo paraventricular anteroventral y el núcleo arcuato (Chemineau *et al.,* 2003; Karsch *et al.,* 1984; Kitahashi y Parhar, 2013; Malpaux *et al.,* 1998, 2002; Sliwowska *et al.,* 2004; Smith *et al.,* 2008). La kisspeptina estimula las neuronas que secretan GnRH que se encuentran principalmente en el área preóptica y en el hipotálamo medio basal. La GnRH es liberada en los capilares del sistema porta-hipofisiario que se encuentra en la eminencia media, y es transportada hasta alcanzar las células gonadotropas de la adenohipófisis donde estimula la secreción de las gonadotropinas LH y FSH (Clarke *et al.,* 2009; Revel *et al.,* 2007; Smith, 2012). Los DC a través de la secreción de melatonina, disminuyen el feedback negativo de la testosterona y del estradiol sobre las neuronas que secretan kisspeptina y GnRH, permitiendo el inicio de la estación sexual en ambos sexos (Chalivoix *et al.,* 2010; Clarke y Karaty, 2013; Kitahashi y Parhar, 2013; Smith *et al.,* 2008; Smith, 2012). En conjunto, estos argumentos sugieren que los machos y hembras caprinas y ovinas poseen un ritmo endógeno de la reproducción que es sincronizado por el fotoperiodo, a través del perfil de secreción de la melatonina.

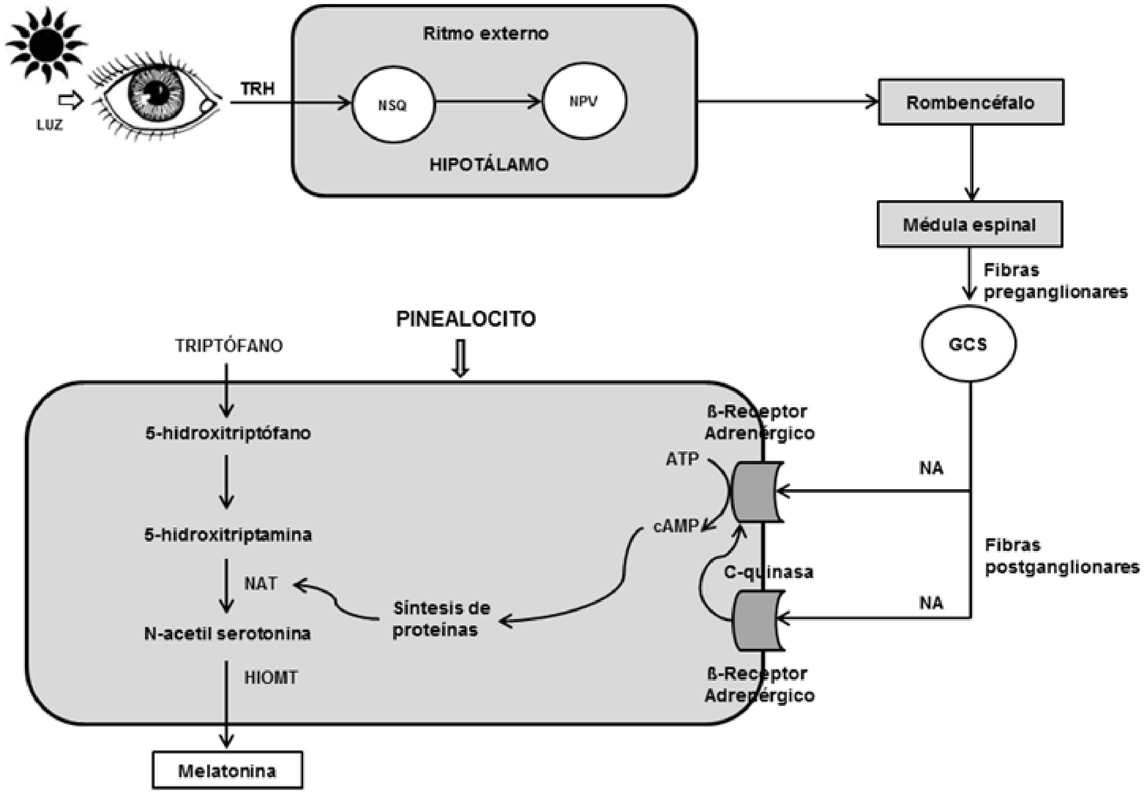

Figura 4. Percepción de la información fotoperiódica y los principales mecanismos del control en la síntesis y secreción de melatonina (Arendt, 1998). **Abreviaciones:** Tracto Retinohipotálamico (TRH), Núcleo Supraquiasmático (NSQ), Núcleo Paraventricular (NPV), Ganglio Cervical Superior (GCS), noradrenalina (NA), Trifosfato de Adenosina (ATP), Monofosfato de Adenosina cíclico (cAMP), N-acetiltransferasa (NAT), e Hidroxiindol-O-Metiltransferasa (HIOMT).

2.1 Técnicas de inducción de la actividad sexual durante el periodo de reposo en los caprinos y ovinos

Actualmente se cuenta con tratamientos “hormonales” que se usan para estimular la actividad sexual en hembras y machos de las especies caprina y ovina durante los meses de reposo sexual: progesterona y/o progestágenos, prostaglandina F2α, gonadotropina coriónica equina (eCG, antes llamada PMSG, gonadotropina de suero de yegua preñada), melatonina y testosterona. Además, existen tratamientos “naturales” que se utilizan con el mismo fin: fotoperiodo artificial y el efecto macho, entre otros (Billings y Katz, 1999; Delgadillo *et al.,* 1992; Duarte *et al.,* 2010; Fierro *et al.,* 2013; Flores *et al.,* 2000; Frank *et al.,* 1945; Pellicer-Rubio *et al.,* 2008).

2.1.2 Tratamientos hormonales

Desde principios de la década de 1960, los dispositivos intravaginales impregnados con progesterona o progestágenos se han usado exitosamente para inducir y sincronizar los ciclos estrales en los pequeños rumiantes (Braden *et al.,* 1960; Corteel, 1975; Leboeuf *et al.,* 2003; Ritar *et al.,* 1984; Robinson, 1965). En las cabras, el protocolo más utilizado para la inducción y sincronización del estro y la ovulación durante el anestro estacional es la inserción intravaginal de esponjas impregnadas con 20 o 45 mg de acetato de fluorogestona, que permanecen en la vagina por un periodo de 11 ± 1 día; 48 horas antes de retirar las esponjas se aplican de 400 a 600 UI de eCG dependiendo de la producción de leche y la estación del año. Adicionalmente se aplican 50 µg de prostaglandina F2α (Corteel *et al.,* 1988; Freitas *et al.,* 1996, 1997; Leboeuf *et al.,* 1998). En estas hembras, la ovulación ocurre alrededor de 24 horas después de retirar la esponja intravaginal (Baril *et al.,* 1996; Leboeuf *et al.,* 2003; Viñoles *et al.,* 2001). Es interesante resaltar que en las cabras existe un protocolo hormonal nombrado como ultra-corto, con una duración de 5 días, y este es usado para inducir, sincronizar el estro y la ovulación. En efecto, el tratamiento consiste en la aplicación de progesterona por medio de un dispositivo intravaginal liberador de progesterona (CIDR, 0.3 g; día 0); adicionalmente, se aplica prostaglandina F2α (160 µg) por vía intra-muscular. Al momento de retirar el CIDR se aplica 250 UI de eCG por vía intra-muscular. En estas hembras, la ovulación ocurre alrededor de 60 horas después de retirar el dispositivo intravaginal (Menchaca *et al.,* 2007; Rubianes y Menchaca, 2003).

En los machos existen también tratamientos hormonales con los que se puede inducir una intensa actividad sexual durante el periodo de reposo. En los machos cabríos de la raza Payoya, la aplicación de 3 implantes subcutáneos de melatonina (18 mg) durante la temporada de reposo sexual en el hemisferio norte (primavera), provoca que la concentración de testosterona sea mayor en los machos que recibieron melatonina que en los machos testigo; y el comportamiento sexual fue más intenso que en los machos testigo (Zarazaga *et al.,* 2010). En carneros, Rosa *et al.* (2000) trataron machos con un implante subcutáneo de melatonina (18 mg) durante la temporada de reposo sexual en el hemisferio norte (mayo-junio), y observaron que los machos tratados mejoraron su comportamiento sexual y fueron más eficientes para estimular la respuesta ovulatoria de las hembras expuestas al efecto macho, que los machos testigo (56% *vs* 24%, respectivamente). Por su parte, Signoret *et al.* (1982) trataron carneros con testosterona (105 mg) durante la temporada de reposo sexual en el hemisferio sur (octubre-noviembre), la respuesta ovulatoria de las hembras durante el efecto macho fue mejor en los machos tratados que desplegaron un intenso comportamiento sexual que aquellos machos testigo que desplegaron un comportamiento sexual débil. En otro estudio realizado por Ungerfeld *et al.* (2014) durante la temporada de reposo sexual en el hemisferio sur (octubre-noviembre), los carneros recibieron 1000 UI de eCG por vía intra-muscular los días -7 y -3 antes del efecto macho. Ellos observaron que la concentración de testosterona fue mayor en los machos tratados con eCG que en los machos testigo (60 nmol/L *vs* 15 nmol/L; respectivamente); además, estos machos fueron más eficientes para estimular la actividad estral de las ovejas que los machos testigo (63% *vs* 38%, respectivamente).

2.1.3 Tratamientos fotoperiódicos

En caprinos y ovinos, los tratamientos fotoperiódicos pueden ser utilizados para inducir la actividad sexual a contra-estación tanto en machos como en hembras (Aboul-Naga *et al.,* 1991; Chemineau *et al.,* 1992b; Delgadillo *et al.,* 1992). La alternancia de periodos de DL artificiales en otoño e invierno seguidos de DC artificiales o naturales, o de un tratamiento con melatonina (que proporciona una señal de DC), permite inducir la actividad sexual de los machos y hembras durante el periodo de reposo sexual estacional (Chemineau *et al.,* 1988b; Delgadillo *et al.,* 1991, 1992, 2002).

2.1.3.1 Tratamientos fotoperiódicos para inducir la actividad sexual en los machos

En los machos cabríos de las razas Alpina o Saanen originarios de latitudes templadas, la exposición a 2 o 3 meses de DL artificiales (16 horas de luz/día) a partir de diciembre seguido del fotoperiodo natural o de la inserción subcutánea de 2 o 3 implantes de melatonina, estimulan la actividad sexual de abril a mayo, durante el periodo de reposo sexual (Chemineau *et al.,* 1992a; Pellicer-Rubio *et al.,* 2007). En los machos cabríos locales de la Comarca Lagunera, un tratamiento con 75 DL artificiales (16 horas de luz/día) a partir del 1 de noviembre seguidos de días naturales (Figura 5) o de la inserción subcutánea de 2 implantes de melatonina, estimulan la secreción de LH, la testosterona, el comportamiento sexual, la producción espermática y el aumento del olor, de febrero a abril, meses que corresponden al periodo de reposo sexual estacional (Delgadillo *et al.,* 2001, 2002; Flores *et al.,* 2000; Rivas-Muñoz *et al.,* 2007; Figura 6). En los machos ovinos de las razas Ile de France y Manech Tête Rousse, la aplicación de 2 meses de DL artificiales a partir de enero o febrero seguido de la inserción de 2 o 3 implantes subcutáneos de melatonina, estimula la actividad sexual a partir de abril o mayo, durante el periodo de reposo sexual (Arranz *et al.,* 1995; Chemineau *et al.,* 1992b).

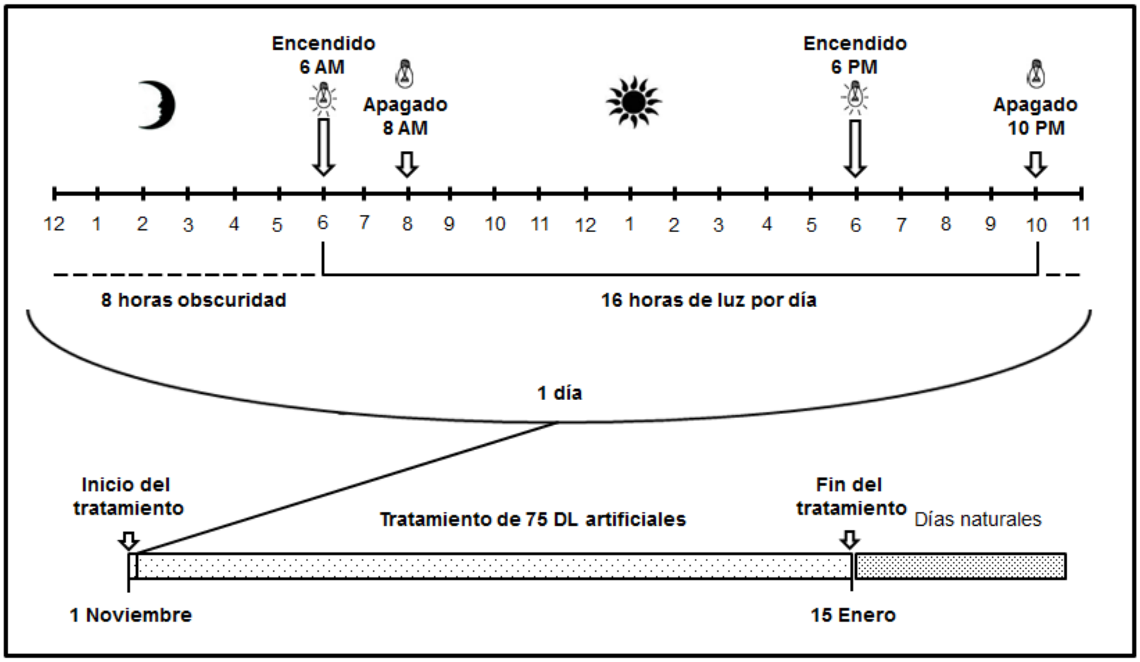

Figura 5. El tratamiento fotoperiódico de 75 DL artificiales a partir del 1 de noviembre aplicado a los machos cabríos de la Comarca Lagunera (26°N). La parte superior muestra la combinación de luz artificial y natural para proporcionar 16 horas luz/día, y 8 horas obscuridad diariamente. La parte inferior muestra los 75 DL artificiales proporcionados durante el tratamiento fotoperiódico (Información tomada de Delgadillo *et al.*, 2002).

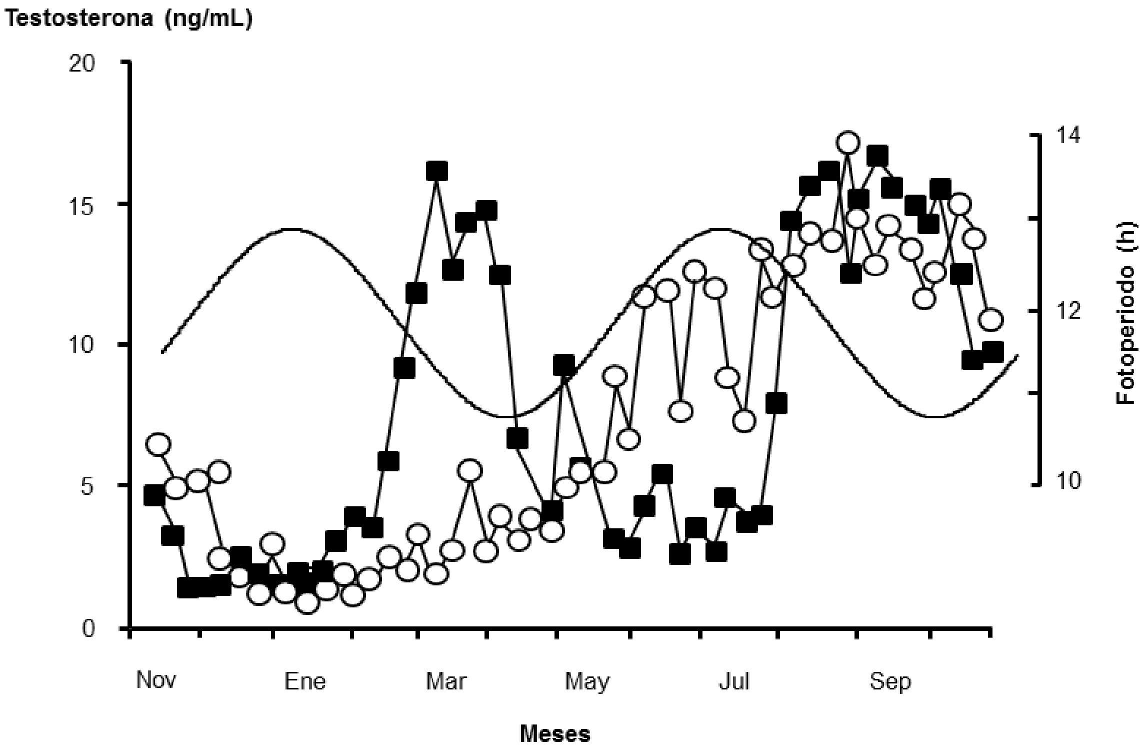

Figura 6. Variaciones de las concentraciones plasmáticas de testosterona de machos cabríos locales de la Comarca Lagunera (26°N) sometidos a cambios naturales del fotoperiodo (testigo;○), o a 75 DL artificiales (16 horas de luz) a partir del 1 de noviembre seguido del fotoperiodo natural (tratado;■, niveles en la escala izquierda), escala derecha representa el fotoperiodo en horas (—; Delgadillo *et al.*, 2002, 2004).

2.1.3.2 Tratamientos fotoperiódicos para inducir la actividad sexual en las hembras

En las hembras caprinas y ovinas, la actividad sexual (estros, ovulaciones) puede ser inducida durante el anestro estacional con tratamientos que alternan DL artificiales seguidos de DC artificiales o naturales, o de un tratamiento con melatonina (Chemineau *et al.,* 1988b, 1992b). En efecto, en las cabras de la raza Payoya expuestas a 3 meses de DL artificiales a partir del 17 de noviembre seguidos del fotoperiodo natural, se indujo la actividad sexual en abril y mayo, durante el periodo de anestro estacional (Zarazaga *et al.,* 2011). De manera similar, en las cabras de la raza Alpina, la exposición a 2 o 3 meses de DL artificiales a partir de enero seguidos de 2 o 3 meses de DC artificiales, días decrecientes naturales o de la aplicación de melatonina por vía oral, subcutánea o intra-muscular, estimulan la actividad sexual de mayo a junio, meses que corresponden al periodo de anestro estacional (Chemineau *et al.,* 1992a). En ovejas de las razas Rahmani y Awassi de Egipto, el estro es inducido durante el anestro estacional utilizando DL artificiales (14 horas de luz/día) en invierno seguidos de DC naturales en primavera (Aboul-Naga *et al.,* 1991, 1992). Estos resultados sugieren que los DL artificiales seguidos de DC artificiales o naturales, o de un tratamiento con melatonina, inducen la actividad sexual fuera de la estación reproductiva en machos y hembras. Además, en hembras caprinas y ovinas, otra alternativa para estimular la actividad sexual durante la época de anovulación estacional es la bioestimulación sexual conocida como el “efecto macho”.

3 La bioestimulación sexual

3.1 Efecto macho

Durante el anestro estacional, la introducción de un macho en un grupo de cabras u ovejas anovulatorias estimula la actividad endocrina, estral y la ovulación en los siguientes 5 días del primer contacto entre machos y hembras (Chemineau, 1987; Delgadillo *et al.,* 2009; Flores *et al.,* 2000; Martin *et al.,* 1980; Shelton, 1960; Underwood *et al.,* 1944). El efecto macho es un fenómeno multisensorial, en el cual intervienen el oído, el tacto, el olfato y la vista, que en conjunto son importantes para obtener una mejor respuesta estral y ovulatoria de las hembras expuestas a los machos (Poindron *et al.,* 1980; Vielma *et al.,* 2008). Los primeros reportes del efecto macho fueron observados en ovejas, estos se registraron en Francia (Girard, 1813; Prudhomme, 1732); mientras que en Australia Underwood *et al.* (1944) encontraron en ovejas de la raza Merino, una correlación entre las fechas de nacimientos de los corderos y la introducción del carnero. Posteriormente, Shelton (1960) lo reportó en cabras. Desde entonces, y hasta la actualidad, el efecto macho en cabras y ovejas se ha utilizado como una alternativa para controlar la reproducción en estas dos especies, debido muy probablemente, a que es una técnica sustentable: limpia, porque no usa hormonas sintéticas; verde, porque no libera residuos potencialmente tóxicos al medio ambiente; y ética, porque contempla el bienestar animal (Delgadillo *et al.,* 2009; Martin y Kadokawa, 2006; Scaramuzzi y Martin, 2008).

3.2 Respuesta endocrina y ovulatoria de las hembras expuestas al efecto macho

En las cabras y ovejas anéstricas, el contacto con un macho puede incrementar inmediatamente la frecuencia de los pulsos de LH, los cuales pasan de 0.3 pulsos en 3 horas antes de la introducción de los machos, a 2.2 pulsos en 3 horas después del primer contacto con éstos (Lindsay *et al.,* 1975; Martin *et al.,* 1986; Poindron *et al.,* 1980; Vielma *et al.,* 2009; Walkden-Brown *et al.,* 1999). En las hembras expuestas al efecto macho, el incremento en la pulsatilidad de la LH se debe al hecho que la presencia del macho estimula las neuronas del núcleo paraventricular anteroventral y del núcleo arcuato que secretan kisspeptina, la cual a su vez activa las neuronas del pulso generador de GnRH, lo que permite la liberación de la LH y FSH (De Bond *et al.,* 2013; Gelez y Fabre-Nys, 2006; Murata *et al.,* 2014; Okamura *et al.,* 2010; Sakamoto *et al.,* 2013; Smith, 2012; Figura 7). Si el contacto entre ambos sexos es permanente y el estímulo del macho persiste, la FSH contribuye en el crecimiento folicular, y en consecuencia, se incrementan los niveles plasmáticos del estradiol secretado por los folículos, desencadenando la conducta estral, el pico pre-ovulatorio de LH y la ovulación, esto ocurre alrededor de 50 horas después de la introducción del macho (Chemineau, 1983, 1987; Ichimaru *et al.,* 1999; Pearce y Oldham, 1988).

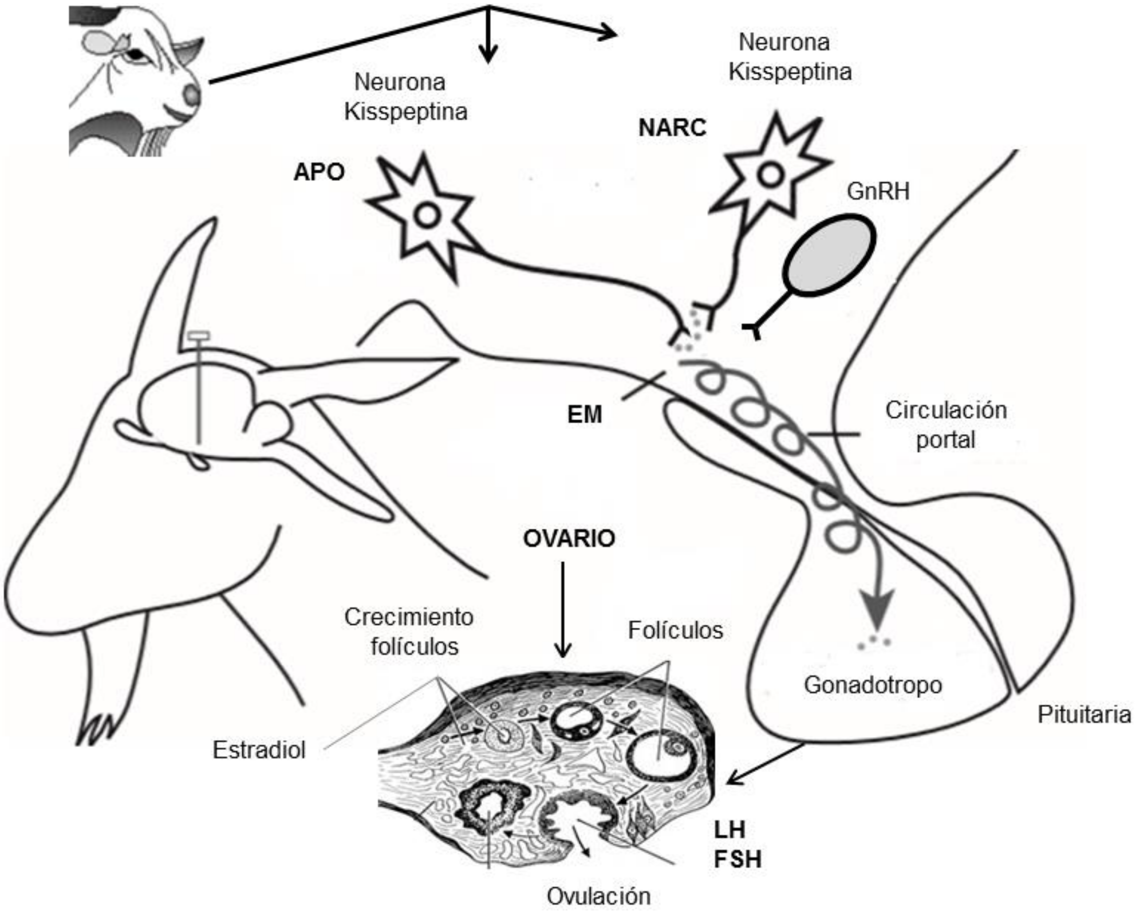

Figura 7. Eje hipotálamo-hipófisis-gónadas. Las neuronas que secretan kisspeptina se encuentran en el núcleo paraventricular anteroventral (NPVAV) y en el núcleo arcuato (NARC). Los axones de las neuronas GnRH alcanzan la eminencia media (EM). Las células que secretan LH y FSH se encuentran en la adenohipófisis de la pituitaria. Las células que secretan estradiol se encuentran en los ovarios (Okamura *et al.,* 2010).

En las cabras, la primera ovulación inducida por la presencia del macho se asocia con una conducta estral en aproximadamente el 60% de los casos; dicha ovulación es seguida por un ciclo estral de corta duración (5 a 7 días) en un 75% de las hembras (Chemineau, 1983; Chemineau *et al.,* 1986). El ciclo estral de corta duración se caracteriza por la presencia de un cuerpo lúteo que secreta poca progesterona (Chemineau *et al.,* 2006; Ott *et al.,* 1980). Posteriormente, se presenta una segunda ovulación, y el cuerpo lúteo es de duración normal; esta ovulación se asocia en el 100% de los casos con conducta estral. Por lo tanto, en esta segunda ovulación la mayoría de las hembras pueden quedar gestantes. Si no hay fecundación durante la segunda ovulación inducida por el macho, 21 días después ocurre una tercera ovulación (Chemineau, 1987). Cuando se introducen los machos, un bajo porcentaje de hembras presentan un ciclo estral de duración normal, en el cual el estro es generalmente acompañado de ovulación (Chemineau, 1983, 1987; Martin *et al.,* 1986).

En las ovejas expuestas a los machos, la primera ovulación es silenciosa, esto quiere decir, sin presencia de estro, y un 50% de las ovejas presentan ciclo ovárico de corta duración (5-6 días; Oldham y Martin, 1978; Poindron *et al.,* 1980). Después de 6 a 9 días de la introducción de los machos, las hembras vuelven a presentar otra ovulación sin mostrar signos de estro. Esta segunda ovulación es seguida por un ciclo ovárico de duración normal y 25 días más tarde las hembras vuelven a presentar estro y ovulación (Chemineau *et al.,* 2006). Estos datos muestran que el estímulo provocado por el macho es capaz de desencadenar la repuesta endocrina, estral y ovulatoria en los primeros 5 días de contacto entre ambos sexos.

4 Factores que pueden modificar las respuestas estral y ovulatoria de las hembras expuestas al efecto macho

En las cabras y ovejas, la respuesta al efecto macho puede ser modificada por varios factores incluyendo la intensidad del comportamiento sexual de los machos, así como el tiempo de contacto entre machos y hembras, entre otros (Bedos *et al.,* 2010; Delgadillo *et al.,* 2001; Perkins y Fitzgerald, 1994; Rivas-Muñoz *et al.,* 2007; Signoret *et al.,* 1982).

4.1 Intensidad del comportamiento sexual del macho

Muchas investigaciones han demostrado que el comportamiento sexual de los machos caprinos y ovinos es un factor importante que determina la respuesta estral y ovulatoria de las hembras expuestas al efecto macho (Delgadillo *et al.,* 2012, 2015; Flores *et al.,* 2000; Perkins *et al.,* 1992; Perkins y Fitzgerald, 1994; Signoret *et al.,* 1982; Vielma *et al.,* 2009). En efecto, cuando los machos despliegan un intenso comportamiento sexual, inducen la ovulación en un mayor número de hembras que aquellos que despliegan un débil comportamiento sexual (Delgadillo *et al.,* 2002, 2015; Flores *et al.,* 2000; Perkins *et al.,* 1992). Por ejemplo, los carneros castrados, tratados con andrógenos que muestran un intenso comportamiento sexual, inducen la ovulación en un mayor número de hembras que aquellos que muestran un bajo comportamiento sexual (71% y 6%, respectivamente; Signoret *et al.,* 1982). En las cabras, el contacto previo de los machos con las hembras en estro mejora significativamente el comportamiento sexual de éstos, y por ende, los machos inducen la ovulación en un mayor número de cabras que los machos que no se expusieron a las hembras en celo y que presentaron un comportamiento sexual débil (Walkden-Brown *et al.,* 1993). De manera similar, los machos cabríos que desplegaron un intenso comportamiento sexual por haber sido sometidos a 75 DL artificiales a partir del 1 de noviembre, estimularon la actividad sexual en más del 90% de las cabras en anestro estacional; en cambio, los machos cabríos no tratados que estaban en reposo sexual, estimularon la actividad sexual solamente del 10% de las hembras (Delgadillo *et al.,* 2002; Figura 8). La capacidad de los machos cabríos foto-estimulados para estimular la ovulación en la mayoría de las cabras se debe, muy probablemente, a su intenso comportamiento sexual, a su fuerte olor y al incremento de las vocalizaciones al estar en contacto con las hembras en anestro (Delgadillo *et al.,* 2002, 2012, 2015; Flores *et al.,* 2000; Rivas-Muñoz *et al.,* 2007). Esta hipótesis es soportada por los resultados de Vielma *et al.* (2009), quienes utilizaron machos foto-estimulados no sedados y sedados. En efecto, los machos foto-estimulados no sedados, que desplegaron un intenso comportamiento sexual, estimularon y mantuvieron elevada la pulsatilidad de LH por 24 horas después del primer contacto con las hembras anéstricas; en cambio, los machos foto-estimulados sedados, que no desplegaron comportamiento sexual, estimularon e incrementaron la pulsatilidad de la LH de las cabras solamente durante las primeras 4 horas de contacto con los machos. Es interesante resaltar que Martínez-Alfaro *et al.* (2014) utilizaron un diseño experimental similar al descrito anteriormente, pero los machos foto-estimulados no sedados y sedados permanecieron 4 días en contacto con las hembras anéstricas: el 95% de las cabras en contacto con los machos no sedados ovularon; en cambio, ninguna ovulación se registró en las hembras en contacto con los machos sedados.

En conjunto, estos resultados sugieren que el comportamiento sexual desplegado por el macho es indispensable para obtener una mayor respuesta estral y ovulatoria en hembras anéstricas, ya que los machos foto-estimulados son más eficientes que los machos testigo para estimular la actividad sexual en las hembras en anestro estacional.

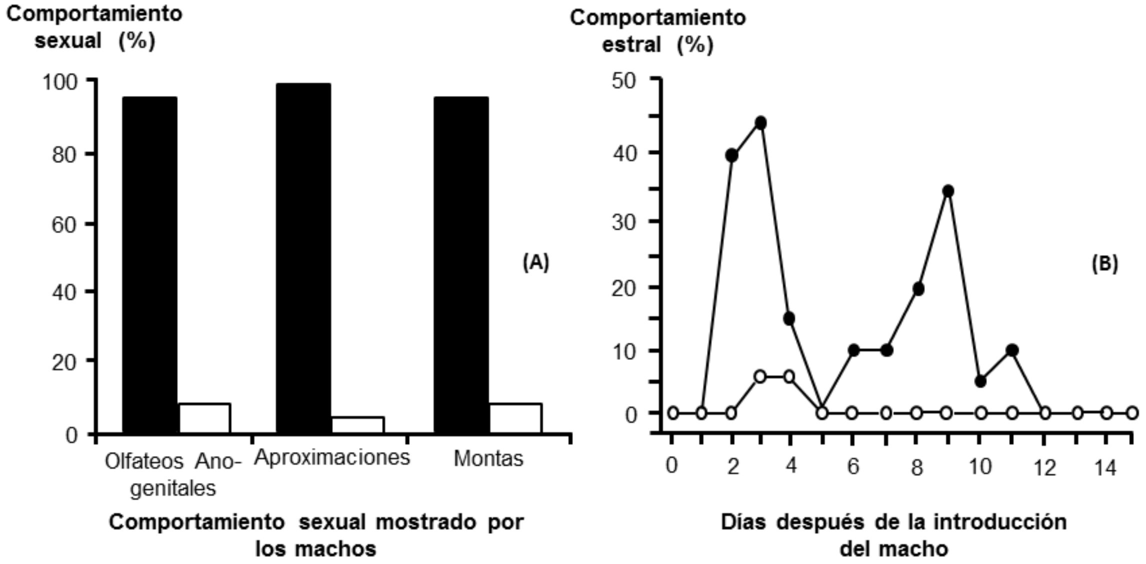

Figura 8. A) Porcentajes de cada tipo de comportamiento observado en los machos en contacto con cabras en anestro estacional: un grupo de machos se sometió al fotoperiodo natural y estaban en reposo sexual (☐), mientras que otros machos se sometieron a 75 DL artificiales a partir del 1 de noviembre, seguidos del fotoperiodo natural, y estaban sexualmente activos (■). **B)** Porcentajes de cabras que presentaron actividad estral al ser puestas en contacto con los machos no tratados, en reposo sexual (○; n=20) o con los machos sometidos a 75 DL artificiales, sexualmente activos (●; n=19). El 100% de las hembras expuestas a los machos tratados presentaron estro, mientras que solo el 10% lo hizo al ser expuestas a los machos en reposo sexual (Delgadillo *et al.,* 2002).

4.2 Tiempo de contacto entre machos y hembras

El tiempo de contacto entre machos y hembras es otra variable que puede modificar la respuesta estral y ovulatoria de las hembras expuestas al efecto macho. Por ello, en los primeros trabajos se reportó que el contacto entre ambos sexos debe ser mantenido por varios días para estimular la ovulación en la mayoría de las hembras (Oldham y Pearce, 1983). En este sentido, Signoret *et al.* (1982) observaron que el porcentaje de ovejas que ovularon se incrementó conforme aumentó el tiempo de contacto entre machos y hembras: 1 día, 18%; 4 días, 51%; 13 días, 61%. En las cabras, el contacto intermitente entre ambos sexos reduce la respuesta ovulatoria de las hembras. En efecto, el 24% de las cabras cashmere ovularon cuando estuvieron en contacto con los machos durante 16 horas diarias por 10 días consecutivos, pero este porcentaje se incrementó al 95% cuando el contacto con los machos se prolongó 24 horas por día durante el mismo periodo de tiempo (Walkden-Brown *et al.,* 1993). Es importante señalar que contrariamente a los resultados descritos anteriormente, Rivas-Muñoz *et al.* (2007) demostraron que la respuesta estral no disminuyó (> 90%) en cabras locales de la Comarca Lagunera cuando se redujo el tiempo de contacto con los machos foto-estimulados de 24 a 16 horas por día durante 18 días consecutivos. Además, recientemente se demostró que el tiempo de contacto entre cabras y machos cabríos foto-estimulados puede reducirse de 16 horas a 1 hora (16, 12, 8, 4, 2 o 1 hora) por día durante 15 días consecutivos, sin disminuir la respuesta ovulatoria de las cabras (100, 100, 100, 94, 100 y 95%, respectivamente; Bedos *et al.,* 2010, 2012, 2014). Bedos *et al.* (2014)

reportaron que las cabras expuestas a los machos de manera continua (24 horas por día) o discontinua (2 horas/día) muestran un perfil de secreción de LH diferente. En efecto, en el grupo de contacto continuo, la secreción de LH se incrementó al introducir los machos y se mantuvo elevado los 4 días que duró el estudio, excepto en aquellas cabras que presentaron un pico pre-ovulatorio de LH. En cambio, en el grupo de contacto discontinuo, la pulsatilidad de LH se incrementó al introducir el macho, pero disminuyó al retirarlo y permaneció baja hasta el día siguiente. La LH se incrementó nuevamente cuando se introdujo el macho. En este estudio no hubo diferencia en los porcentajes de hembras que ovularon al permanecer de manera continua o discontinua con los machos (Bedos *et al.,* 2014).

III. PLANTEAMIENTO DEL PROBLEMA

Como se revisó en la literatura, existe evidencia que un tratamiento de 75 DL artificiales a partir del 1 de noviembre, estimula la actividad sexual de los machos locales de la Comarca Lagunera en marzo y abril, meses que corresponden al periodo de reposo sexual (Delgadillo *et al.,* 2001, 2015; Flores *et al.,* 2000; Rivas-Muñoz *et al.,* 2007). Durante estos meses, los machos foto-estimulados despliegan un intenso comportamiento sexual y muestran ser muy eficientes para estimular la ovulación en la mayoría de las cabras expuestas a ellos (Delgadillo *et al.,* 2002; Flores *et al.,* 2000; Rivas-Muñoz *et al.,* 2007). Sin embargo, hasta hoy no hay reportes en la literatura que indiquen que el tratamiento con DL en los machos cabríos locales de la Comarca Lagunera pueda reducirse a menos de 75 días, sin que esto disminuya la respuesta endocrina y sexual de los mismos. Tampoco se conoce si los machos que son sometidos a menos de 75 DL artificiales son capaces de inducir la actividad ovulatoria de las cabras durante el anestro estacional.

Por otra parte, en los ovinos, el porcentaje de hembras que ovulan depende del tiempo que permanecen en contacto con los machos. Este porcentaje aumenta del 18% al 61% cuando la duración de contacto en días se incrementó de 1 a 13 días, respectivamente (Signoret *et al.,* 1982). En las cabras locales de la Comarca Lagunera, el porcentaje de hembras que ovulan no disminuye cuando son expuestas a los machos durante solo algunas horas al día (16, 12, 8, 4, 2 o 1 horas; Bedos *et al.,* 2010, 2012, 2014). En las cabras de la Comarca Lagunera, no existen datos que describan la respuesta ovulatoria al exponerlas a los machos foto-estimulados por 1, 5, o 10 días.

IV. OBJETIVOS

1. Determinar si la disminución de los tratamientos de DL a 45, 30, o 15 días, no reduce la respuesta sexual de los machos cabríos al tratamiento, asimismo, determinar si los machos foto-estimulados con 45 o 30 DL inducen la actividad ovulatoria en las cabras anéstricas.

2. Determinar si la disminución del tiempo de contacto entre machos y hembras no reduce la respuesta ovulatoria de las cabras cuando son expuestas a los machos foto-estimulados por 1, 5, o 10 días.

V. HIPÓTESIS

1. La disminución de los tratamientos de DL a 45, 30, o 15 días no reduce la respuesta sexual de los machos cabríos al tratamiento, ni su capacidad para inducir la actividad ovulatoria en las cabras anéstricas.

2. La disminución del tiempo de contacto entre machos y hembras no reduce la respuesta ovulatoria de las cabras cuando son expuestas a los machos foto-estimulados por 1, 5, o 10 días.

VI. ARTÍCULOS

Estudio 1

Artículo 1. Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of male goats to stimulate ovulatory activity in seasonally anovulatory females.

Publicado: Domestic Animal Endocrinology 48 (2014) 119–125.

Estudio 2

Artículo 2. One day of contact with photo-stimulated bucks is sufficient to induce ovulation in seasonally anestrous goats.

Publicado: Theriogenology (doi:10.1016/j.theriogenology.2015.05.019).

Estudio 1

Artículo 1. Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of male goats to stimulate ovulatory activity in seasonally anovulatory females.

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Reducing exposure to long days from 75 to 30 days of extra-light treatment does not decrease the capacity of male goats to stimulate ovulatory activity in seasonally anovulatory females

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ABSTRACT

The response of male goats exposed to different durations of long days (LD) during an extra-light treatment in autumn-winter, and their ability to induce ovulations in seasonally anovulatory goats were investigated in 2 experiments. In experiment 1, control males were exposed to natural photoperiod (n = 5), whereas 4 additional groups (n = 5/group) were exposed to 16 h of light per d during 75, 45, 30, or 15 d of LD. In the 4 groups, photoperiodic treatments ended on January 15th. Plasma concentrations of testosterone were determined in blood samples obtained once a week from October 15th to May 30th. The rise of testosterone levels occurred earlier in males from the 75-LD and 45-LD groups than in those from the 30-LD, 15-LD, and control groups ($P < 0.05$). In addition, the time during which levels of testosterone remained >5 ng/mL was longer in males from the 75-LD and 45-LD than in those from the 30-LD and 15-LD groups ($P < 0.05$). In experiment 2, a group of anovulatory goats (n = 13) was isolated from males, while 3 additional groups were put in contact during 15 d with males previously exposed to 75, 45, or 30 days of LD (n = 25, 27, and 26 females/group, respectively and n = 3 males per group). The proportion of goats that ovulated was higher in the 3 groups in contact with the photo-stimulated males (range: 88%–92%) than in the group isolated from them (0%; $P < 0.05$). The proportion of pregnant females did not differ between the 3 groups of does in contact with photo-stimulated males (range: 78%–92%; $P > 0.05$). We conclude that, in our experimental conditions, a photoperiodic treatment as short as 30 d of LD during autumn-winter, stimulated testosterone secretion of bucks during their period of sexual rest and rendered them able to induce ovulations in seasonal anestrous goats and to obtain pregnancies in these females.

1. Introduction

The reproductive seasonality showed by breeds of goats from temperate and subtropical latitudes constitutes an important limitation for inducing reproduction during the season of sexual rest [1–4]. To solve these limitations associated with reproductive seasonality, socio-sexual relationships have been used to stimulate the sexual activity of seasonal breeds of goats during the nonbreeding season [5–7]. Thus, the introduction of a male into a group of seasonally anestrous does or ewes, stimulates and synchronizes LH secretion, ovulations, and estrous behavior

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[8–10]. This technique of sexual biostimulation is known as the “male effect” [11–13].

The proportion of females ovulating or displaying estrous behavior after the male effect varies according to the intensity of the sexual behavior of males [14,15]. Indeed, in the northern hemisphere, bucks rendered sexually active by exposure to 75 d of long days (LD) from November 1st followed by natural photoperiod, induce ovulations in most of seasonally anovulatory females, whereas untreated bucks are unable to do so [16,17]. The capacity of the photo-stimulated males to induce the ovulatory activity in anestrous females is likely because of the fact that the 75 d of LD stimulates testosterone secretion, sexual behavior, odor, and male vocalizations from February to April, these months corresponding to the nonbreeding season in the northern hemisphere [17–19].

In rams kept in a lightproof building, a period of 30 d of LD followed by 120 d of short days (SD) stimulated testosterone secretion and increased scrotal circumference [20]. Moreover, bucks and rams exposed to alternate long and short day-light regimens of 30-d duration fail to exhibit endocrine and reproductive changes expected for males exposed to natural photoperiod [21–23]. In Alpine bucks exposed to the previously described light regime, testosterone secretion increased during LD and decreased during SD because of the rapid alternations of day length [23]. All these results suggest that in bucks and rams, 30 d of LD are long enough to sensitize the males and allowing them to be responsive to the following 30 artificial days of SD. However, the minimal duration of artificial LD necessary to induce the sexual activity of bucks during the natural sexual rest when kept under natural photoperiodic conditions is not known, and this is also the case regarding the capacity of these photoperiodic treated males to stimulate the ovulatory activity in seasonally anestrous goats. Thus, we performed 2 experiments: in the first one, we compared testosterone secretion in bucks exposed to 15, 30, 45, and 75 d of LD and hypothesized that 15 d of LD would be able to stimulate testosterone secretion. Then, in a second experiment, we compared the response to the male effect in seasonally anovulatory goats exposed to males submitted to 30, 45, and 75 d of LD.

2. Materials and methods

2.1. General management conditions

We performed 2 experiments during 2 consecutive years using local Mexican goats (*Capra hircus*) from the Laguna region in the State of Coahuila, Mexico (latitude, 26°23′ N and longitude, 104°47′ W). In these females isolated from males, the anestrous period lasts from February-March to August-September, while in bucks isolated from females, the sexual rest lasts from January-February to May-June [3,4]. In the present experiments, animals were fed 2 kg of alfalfa hay (18% CP) and 200 g/d of commercial concentrate (14% CP; 1.7 Mcal/kg) per animal, with free access to mineral blocks and water during the study.

Experimental procedures used in both experiments were in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [24].

2.2. Experiment 1

The objective of experiment 1 was to determine whether durations of LD treatments as short as 15 d could stimulate the testosterone secretion of bucks during the nonbreeding season.

2.2.1. Photoperiodic treatments

Male goats were 1-yr-old at the beginning of the study. On October 1st, males were divided into 5 groups (n = 5/group) balanced for scrotal circumference (Table 1). Bucks from each group remained into a shaded outdoor pen (5 × 5 m) until the end of the study on May 30th. Control males were exposed to the natural photoperiodic conditions (13 h 41 min of light at the summer solstice and 10 h 19 min of light at the winter solstice) throughout the study. Males from the other 4 groups were submitted to different periods of LD, all ending on January 15th (Fig. 1). Long days

Table 1
Characteristics of testosterone secretion in local male goats from subtropical Mexico (26°N). Control males were exposed to natural photoperiodic conditions (n = 5). The other 4 groups were exposed to artificial long days (LD; 16 h of light per d) during 15 (15-LD), 30 (30-LD), 45 (45-LD), and 75 (75-LD) consecutive days. The onset of photoperiodic treatments differed between groups but all of them ended on January 15th.

Groups	n	First increase in plasma testosterone >5 ng/mL	First decrease in plasma testosterone <5 ng/mL	Duration of high plasma testosterone >5 ng/mL (d)	Interval between end of LD and first increase in plasma testosterone >5 ng/mL (d)	Scrotal circumference (cm)
Control	5	May 16 ± 4^{a}	—	—	—	22 ± 0.3^{a}
15-LD	5	March 28 ± 5^{b}	April 26 ± 2^{a}	29 ± 3^{a}	72 ± 5^{a}	23 ± 0.4^{a}
30-LD	5	March 20 ± 3^{b}	April $17 \pm 2^{a,b}$	28 ± 3^{a}	64 ± 3^{a}	23 ± 0.2^{a}
45-LD	5	February 17 ± 1^{c}	April 7 ± 3^{b}	49 ± 4^{b}	33 ± 1^{b}	24 ± 0.6^{a}
75-LD	5	February 14 ± 4^{c}	April 23 ± 4^{a}	69 ± 6^{c}	30 ± 4^{b}	23 ± 0.3^{a}

Abbreviation: SEM, standard error of the mean.
Results are expressed as mean ± SEM.
^{a,b,c} Values with different letters within each column differ significantly ($P < 0.05$).

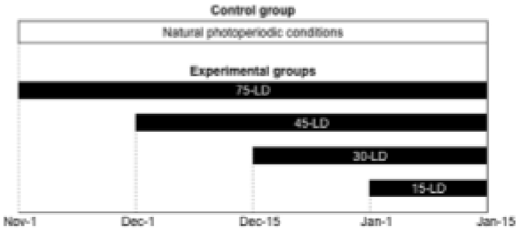

Fig. 1. Schedule of the photoperiodic treatments used to stimulate the sexual activity of local male goats from subtropical Mexico (26°N) during the natural sexual rest. Control males were exposed to natural photoperiodic conditions in an open pen (n = 5). The other 4 groups were kept in open pens and subjected to artificially long days (LD; 16 h of light per d) during 75 (75-LD), 45 (45-LD), 30 (30-LD), and 15 (15-LD) consecutive d (n = 5 per group). The onset of the photoperiodic treatments differed between groups but all of them ended on January 15th. After the end of long-day treatments, all bucks were placed under natural photoperiodic conditions until the end of experiment on May 30th.

lasted 16 h and were obtained providing natural light with additional artificial light from 6 AM to 8 AM and from 6 PM to 10 PM. Intensity of artificial light was at least 300 lx at the level of the eyes of the animals.

2.2.2. *Measurements*

Plasma testosterone concentrations were determined in blood samples obtained at 9 AM once a week throughout the study.

2.2.3. *Blood sampling*

Blood samples were obtained by jugular venipuncture in 5-mL tubes containing heparin. After collection, samples were centrifuged at 2500× *g* for 20 min and plasma was stored at −15°C until determination of hormone concentrations. Testosterone was determined by RIA according to the technique described by Hochereau-de-Reviers et al [25]. Sensitivity was 0.3 ng/mL. The intra-assay CV was 6%.

2.3. *Experiment 2*

Considering that in experiment 1, testosterone secretion of males exposed to 15 and 30 d of LD was similar, the objective of experiment 2 was to determine whether males treated with only 30 d of LD could stimulate the ovulatory activity in seasonally anovulatory goats.

2.3.1. *Males*

Males were 2-yr-old at the beginning of the photoperiodic treatments. On October 15th, 9 males were randomly divided into 3 groups (n = 3/group) and remained into a shaded outdoor pen (5 × 5 m) until the end of the study on April 19th. Males from each group were submitted to 75, 45, or 30 d of LD as indicated in Figure 1. In the present study, the response of anestrous goats to the presence of untreated control bucks was not investigated because several previous studies have shown that untreated bucks do not induce a significant reproductive response in anestrous does. Indeed, Flores et al [15] reported that only 2 females of 34 ovulated following the introduction of untreated bucks and no response at all was found (0/20) in another study by Delgadillo et al [16]. Similar results were also reported in another study, with only 1/22 females ovulating following a male effect with untreated bucks [26]. Furthermore, the fact that untreated bucks fail to induce a significant reproductive response in does is also supported by the fact that even in the presence of estrous females in the group at the moment of the male effect, untreated bucks did not induce a significant response of anestrous females as none of them (0/20) displayed estrus [27].

2.3.2. *Females*

We used multiparous anovulatory goats that had given birth between September 1st and October 30th, and kids had been weaned between 20 and 30 d of age. All females were isolated from males from December 15th until April when exposure to males was implemented (see section 2.3.3). On March 23th and 29th, each female was submitted to a transrectal ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe to verify their anovulatory condition. On March 29th, anovulatory females were divided into 4 groups balanced for body condition score (Table 2), and all groups were kept in separate shaded open pens (6 × 4 m). The distance between each of the 4 groups of females was more than 300 m, thus preventing any risk of interference by the treatments between groups.

2.3.3. *Male effect*

On April 2nd at 8 AM (d 0), females were exposed to randomly selected light-treated bucks (n = 3 per group). One group was exposed to the males subjected to 75 d of LD (75-LD group; n = 25); the second one was exposed to males

Table 2
Ovulatory response of anestrous female goats exposed to males (n = 3 per group) rendered sexually active by exposure to artificial long days (LD; 16 h of light per d) during 30 (30-LD), 45 (45-LD), and 75 (75-LD) consecutive days. The onset of photoperiodic treatments differed between groups but all of them ended on January 15th. Control females remained isolated from males.

Groups	n	BCS[a] (mean ± SEM)	Females with ovulations (%)[b]	Ovulation rate (mean ± SEM)	Pregnancy rate (%)[c]
Control	13	1.9 ± 0.1[d]	0	—	—
30-LD	26	1.8 ± 0.1[d]	88[d]	1.5 ± 0.1[d]	22/26 (85)[d]
45-LD	27	1.8 ± 0.1[d]	89[d]	1.6 ± 0.1[d]	21/27 (78)[d]
75-LD	25	1.7 ± 0.1[d]	92[d]	1.7 ± 0.1[d]	23/25 (92)[d]

[a] BCS, body condition score (range: 1, very lean; 4, fat).
[b] Percentages of females with ovulations and ovulation rates were detected by transrectal ultrasonography 17 d after the introduction of males.
[c] Percentages of pregnancy rates were detected by abdominal ultrasonography 50 d after the introduction of males.
[d] Values with same letters within each column do not differ significantly ($P > 0.05$).

subjected to 45 d of LD (45-LD group; n = 27) and the third one was exposed to males subjected to 30 d of LD (30-LD group; n = 26). Each group was divided in 3 subgroups. In each subgroup, one male was in contact with 8 to 9 females. Within each group, males were rotated daily between subgroups to avoid possible individual effects of bucks. Males remained with females for 15 d. The control group (n = 13) was isolated from males and was not divided in subgroups. To ensure that bucks were sexually active, their sexual behavior was observed individually by trained observers from 8 AM to 9 AM on d 0, 1, and 8 following introduction of bucks in the groups of females. Nudging, anogenital sniffing, flehmen response, self-urination, mounting attempts, and mounting with intromission were recorded [15,19]. Monitoring of sexual activity showed that all males displayed some sexual activity in the 3 groups (Fig. 2).

2.3.4. Measurements

The presence of male-induced ovulations and ovulation rates were assessed by the presence and number of corpora lutea 17 d after introduction of the bucks, observed in each female by transrectal ultrasonography with the same equipment described previously [28,29]. Pregnancy rates were determined by abdominal ultrasonography 50 d after exposure to males using the same device described previously connected to a 3.5 MHz abdominal probe.

2.4. Statistical analyses

In experiment 1, testosterone plasma concentrations were analyzed by a 2-way analysis of variance with repeated measurements to test effects of photoperiodic treatment and time of experiment. When interactions between treatment and time existed, pairwise comparisons among groups were performed to test the treatment effects within time followed by *t* test for individual time point's comparisons among groups.

In experiment 2, the proportions of females that ovulated and pregnancy rates were compared by χ^2. Ovulation rates were compared using the Kruskall–Wallis and U Mann–Whitney tests. Data are presented as mean ± SEM. The statistical analysis was carried out using the statistical package SYSTAT 13 [30].

3. Results

3.1. Experiment 1

3.1.1. Testosterone secretion

There was a treatment by time interaction indicating that changes in testosterone release differed between groups ($P < 0.0001$; Fig. 3). The increase of testosterone plasma concentrations occurred earlier in the 75-LD and 45-LD groups than in 30-LD and 15-LD groups ($P < 0.05$; Table 1). In addition, duration of high testosterone secretion (>5 ng/mL) was longer in males from the 75-LD and 45-LD than in those from the 30-LD and 15-LD groups ($P < 0.05$).

3.2. Experiment 2

3.2.1. Ovulatory response and pregnancy rates of females exposed to photoperiodic-treated males

The proportion of goats that ovulated was greater in those in contact with photo-stimulated males than in isolated females ($P < 0.0001$). In contrast, the proportion of females that ovulated, ovulation and pregnancy rates did not differ between groups of females in contact with LD-treated males regardless of the duration of the LD-treatments ($P > 0.05$; Table 2).

4. Discussion

Our results show that testosterone secretion of male goats was induced during the season of sexual rest by exposure to 15 d of LD followed by natural photoperiodic conditions and

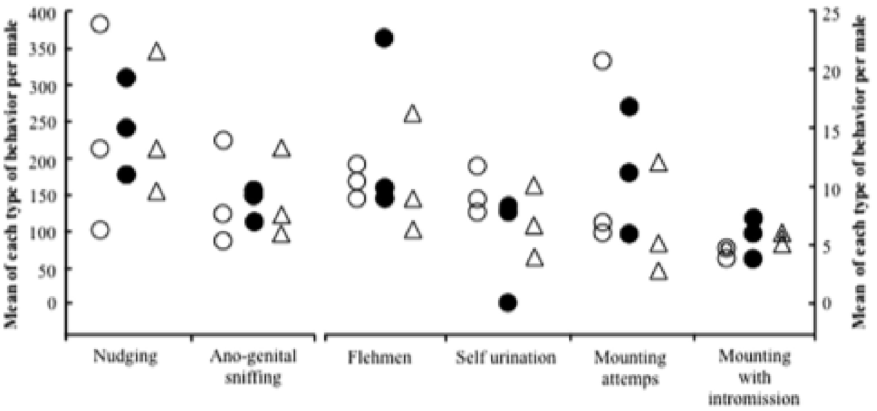

Fig. 2. Individual values of occurrences of nudging, anogenital sniffing, flehmen response, self urination, mounting attempts, and mounts with intromission in bucks (n = 3/group) interacting with anestrous seasonal goats. Sexual behavior of males was observed from 8 AM to 9 AM on d 0, 1, and 8 following their introduction in the groups of females. Males were previously subjected to artificially long days (16 h of light per d) during 75 (○), 45 (●), or 30 (Δ) consecutive d. The onset of the photoperiodic treatments differed between groups but all of them ended on January 15th. After the end of long-days treatments, all bucks were placed under natural photoperiodic conditions until they were removed from the does' groups on April 17th.

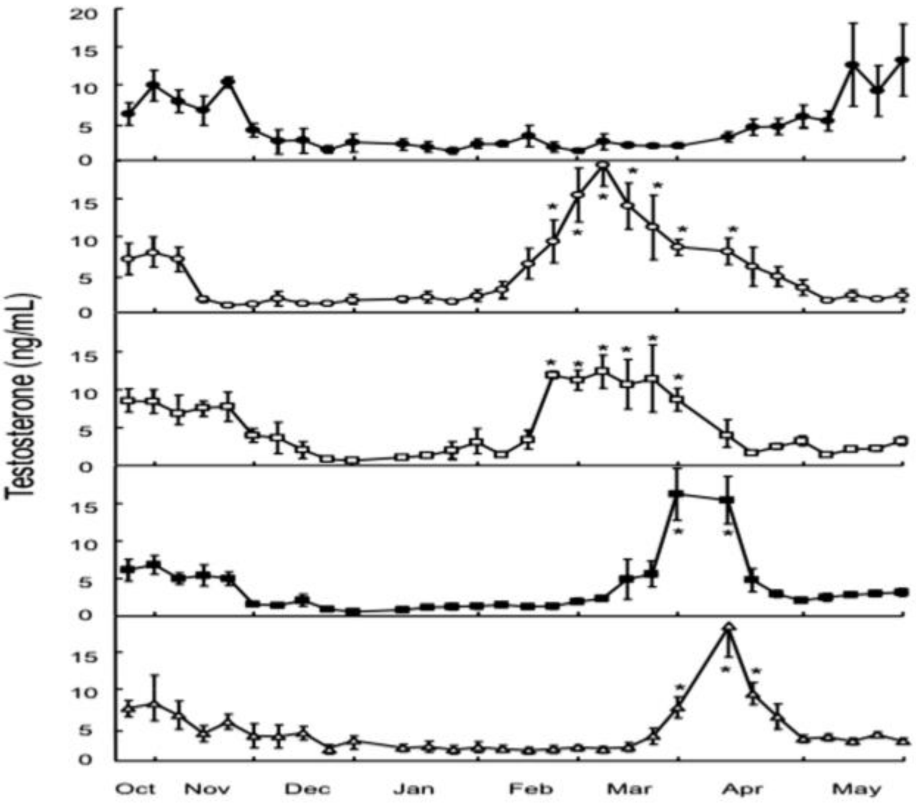

Fig. 3. Variations of plasma testosterone concentrations (mean ± SEM) in male goats from subtropical Mexico (26°N). Control males were exposed to natural photoperiodic conditions (●; n = 5). The other 4 groups were subjected to artificially long days (16 h of light per d) during 75 (○), 45 (□), 30 (■), and 15 (Δ) consecutive days (n = 5 per group). The onset of the photoperiodic treatments differed between groups, but all of them ended on January 15th. After the end of long-day treatments, all bucks were placed under natural photoperiodic conditions until the end of experiment on May 30th. Stars indicate significant differences between the photoperiodic-treated males when compared with control males (* $P < 0.05$). SEM, standard error of the mean.

that 30 d of LD were long enough to make males able to stimulate the ovulatory and reproductive activities in seasonal anestrous goats. These results showed, for the first time, that reducing duration of LD-treatments did not decrease the ability of males to induce fertile ovulations in seasonal anestrous goats exposed to the photo-stimulated bucks.

In experiment 1, our results strongly suggest that the onset of perception of LD timed the increase of testosterone secretion in the photoperiodic-treated bucks: the earlier perception of LD advanced the increase of testosterone secretion. In ewes, it was shown that perception of LD in spring is a critical signal in the timing of the onset of the breeding season [31–34]. In male goats, it is probable that perception of LD is operating in the same way as in sheep in timing the onset of breeding season. Indeed, in Alpine bucks exposed to 75 d of LD from December 1st, plasma testosterone concentrations increased 3 wk later than in bucks exposed to the same duration of LD from November 1st [35,36]. In our present study, the response of the photoperiodic treatments with LD was significantly delayed when starting on December 15th onward as observed in the 30-LD and 15-LD groups. Our results are similar of those reported in ewes, in which ovulatory activity was delayed in females exposed to LD from December compared with those perceiving LD from November [33]. Thus, we can conclude that the earlier exposure to LD advanced the timing of the reproductive rhythm in subtropical male goats.

Duration of high plasma levels of testosterone concentrations differed according to the length of LD-treatments. In our study, all LD-treatments ended on January 15th, avoiding males perceiving a different SD signal after the end of LD-treatments. This fact strongly suggests that the difference in the duration of the breeding season observed in our photoperiodic-treated males was the consequence of the duration of LD-treatments and not to a different signal of SD, contrary to what was reported in sheep [37]. Interestingly, in our study, the effect of the duration of LD treatments on testosterone secretion may not be continuous. Rather, it may exist a threshold situated between 30 and 45 d of LD after which the males responded by a robust increase and prolonged in testosterone secretion. Thus, our results suggest that in bucks, as well as in rams [20], the length of LD determines the duration of high testosterone plasma concentrations.

In our present study, the sperm production of experimental bucks was not determined to avoid a possible influence of the presence of the artificially-induced estrus goats on the sexual behavior and/or semen production of bucks [38,39]. However, in bucks and rams kept under natural photoperiodic conditions or submitted to photoperiodic treatments to induce their sexual activity during the sexual rest season, the increase of circulating levels of testosterone precedes the elevation of testicular weight, a reliable index of quantitative and qualitative sperm production. Sperm production and/or the intensity of sexual behavior decreased at the same time than plasma testosterone levels [40,41]. Then, in our present study, we can predict, considering the patterns of testosterone secretion, that the quantitative and qualitative sperm production was high at least during the time that testosterone remained elevated.

In experiment 2, we showed that males exposed to 45 and 30 d of LD were able to stimulate the ovulatory and reproductive activities in anestrous goats, as did those from the 75-LD group. Male sexual behavior is known to be an important element to stimulate the ovulatory activity in seasonal anestrous goats and ewes [10,14,15,42] and, in the present study, male sexual activity was present in the 3 groups. Therefore, it is possible that the sexual behavior displayed by the photo-stimulated bucks was strong enough to induce ovulations when the male effect was performed, even with males exposed to 45 or 30 d of LD. Further experiments with a larger number of males per group would be necessary to confirm this point. In any case, exposing bucks to only 45 or even 30 d of LD was sufficient to obtain a good ovulatory response and most goats became pregnant at similar rates as in other experiments [15,19,43]. These results indicate that 30 LD were long enough to stimulate and maintain spermatogenesis for normal fertilization when the male effect was performed, even though quantitative and qualitative sperm production of photo-stimulated bucks was not determined in our experiment.

We conclude that in our experimental conditions, bucks receiving a photoperiodic treatment as short as 30 d of LD during autumn-winter can be used for stimulating the female does because it stimulated testosterone secretion of bucks during their sexual rest and rendered them able to induce ovulations and to obtain pregnancies in anestrous seasonal goats. The shortness of this treatment renders the preparation of the bucks easier and makes therefore its application more flexible. Its shortness also allows to a group of farmers to share the mobile electric apparatus necessary to pen the bucks during the photoperiod treatment and to reduce the direct electric consumption, which are the 2 main external costs of the treatment, thus reducing by more than half the total cost of the treatment.

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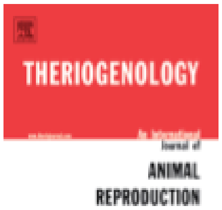
Estudio 2

Artículo 2. One day of contact with photo-stimulated bucks is sufficient to induce ovulation in seasonally anestrous goats.



One day of contact with photo-stimulated bucks is sufficient to induce ovulation in seasonally anestrous goats

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“Revised”

One day of contact with photo-stimulated bucks is sufficient to induce ovulation in seasonally anestrous goats

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Abstract

In seasonally anovulatory goats, daily contact with photo-stimulated bucks for 15 days stimulates ovulations in most females. In this study we determined whether ovulation could be induced in goats exposed to photo-stimulated bucks for less than 15 days. Bucks were rendered sexually active during the non-breeding season by exposure to 2.5 months of long days from November 1st. The control group of females was exposed to one photo-stimulated buck for 15 days (n = 12). Other three experimental groups were exposed to males (n = 1 per group) for 1, 5 or 10 days (n = 14 or 15 females per group). Ovulations were determined by measurement of daily plasma progesterone concentrations during 17 days. All females from the control and experimental groups ovulated at least once during the experiment ($P > 0.05$). Furthermore, the proportions of goats that displayed a short luteal phase followed by a new ovulation, or a normal luteal phase after being in contact with males, did not differ between groups depending on the duration of time of contact with the photo-stimulated males ($P > 0.05$). In contrast, the proportions of females that displayed a short luteal phase followed by anovulation was greater in goats in contact with males for 1 day, than in those in contact with males for 10 and 15 days ($P < 0.05$), whereas it did not differ from females exposed to males for 5 days ($P > 0.05$). We conclude that 1 day of contact with sexually active males is long enough to stimulate the ovulatory activity in seasonally anovulatory goats. However, a significantly higher proportion of females exposed to males for 1 day did not ovulate again after showing a short luteal phase.

Keywords: Caprine, Male effect, Photoperiod, Reproductive seasonality, Duration of contact, Ovulation

1. Introduction

The sociosexual relationships, particularly the "male effect", can be used to stimulate the activity of the hypothalamo-pituitary-ovarian axis in goats and ewes during the seasonal anestrus [1,2,3]. Indeed, the introduction of a male into a group of anovulatory females can induce and synchronize LH secretion, ovulations and estrous behavior in the following 5 days [4,5,3]. In goats, the response of females exposed to males involves an immediate increase of LH secretion that culminates in a preovulatory surge leading to ovulation [6,7,8]. A variable number of females show estrous behavior at the first induced ovulation 2–5 days after male introduction. Most goats have a short ovarian cycle of 5–7 days of duration, followed by a second ovulation, which usually occurs around 7–10 days following the introduction of the male and which is associated with estrous behavior and followed by a luteal phase of normal duration [9,10].

The proportions of females that ovulated vary according to the intensity of the sexual behavior displayed by males to which they are exposed [11,12,13]. Indeed, most of goats or ewes ovulated and showed estrous behavior when they were exposed to bucks or rams displaying an intense sexual behavior, as opposed to those in contact with males displaying a weak sexual behavior [13,14,15]. In goats, for example, most of does (>90%) ovulated when they were exposed to bucks rendered sexually active by exposure to long days in autumn-winter, whereas a low proportion of females (<10%) did so when exposed to those in seasonal rest [11].

Duration of contact period between males and females is another factor involved in the ovulatory response of females exposed to males, probably because LH secretion decreased when males are removed from females, preventing ovulation to occur [16]. In fact, only 18% of ewes ovulated when exposed to wethers treated with testosterone for 1 day, but this percentage increased to 53% and 61% when the males were maintained with females for 4 or 15 consecutive days, respectively [17]. Similarly, in cashmere goats, only 24% of females ovulated when exposed to males for 16 h per day during 10 days, but this percentage increased to 95% when bucks remained with females 24 h per day during the same duration [18]. In contrast to the above-described results, in local goats from subtropical Mexico, most of goats (>92%) displayed estrous behavior when the time of contact with males was reduced from 24 hours to 16 hours per day during 18 days [14]. Interestingly, recent studies indicate that most of Mexican goats ovulated and became pregnant when daily contact between photo-stimulated males and females was further reduced from 24 hours to 4, 2 or even 1 hour for 15 days [6,19,20]. The different results described in ewes *versus* Cashmere and Mexican goats, can be explained by the fact that Rivas-Muñoz et al. [14] and Bedos et al. [6,19,20] used males rendered sexually active by exposure to a long-day photoperiodic treatment [11,21], whereas Signoret et al. [17] and Walkden-Brown et al. [18] used photoperiodic-untreated males, which were in seasonal sexual rest. In fact, the high level of sexual behavior, the intense odor and vocalizations showed by bucks induced by long-days treatments are important cues to induce endocrine and reproductive activities in anestrous goats [7,14,22]. Considering the high proportions of goats that ovulated when exposed to sexually active bucks for a short duration, we set the hypothesis that sexually active males could be able to induce ovulations in goats when permanent daily (24 hours/day) contact between sexes is less than 15 days.

2. Materials and methods

2.1. Ethical note

The experimental procedures used in the current experiment were in accordance with the Official Mexican Rule for the technical specifications for the production, care and use of laboratory animals [23].

2.2. *Location and general management conditions of animals*

The current experiment was performed in the Region of Laguna, in the State of Coahuila, Mexico (latitude, 26°23 ′N and longitude, 104°47 ′W). In female local goats, the anestrous season lasts from February-March to August-September [24], whereas in bucks, the sexual rest lasts from January-February to May-June [25]. 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 with 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate (14% CP; 1.7 Mcal/kg) with *ad libitum* access to water during the study.

2.3. Management of experimental groups

Multiparous anovulatory goats used in the present experiment were 2-3 yr old at the beginning of experiment. On March 15th and 21st, each female was submitted to a transrectal ultrasonography using an Aloka SSD-500 scanner connected to a transrectal 7.5 MHz linear probe to determine their ovarian cyclicity [26,27]. Taking into account the duration of follicular phase in does [28], this observation, associated with the measurement of progesterone concentration in a blood sample taken immediately before introduction of males (see below) is efficient for the determination of the anovulatory state of these females [19,29]. There was not any doe cycling before introduction of males, which is indicative of deepth of anestrus [4]. On April 1st, females were assigned to four groups balanced for body condition score (average BCS between 2 and 2.2 on a scale ranging from 1, very thin, to 4, very fat [30; Table 1]. These four groups remained in shaded open pens under natural day length during 15 days, and were exposed to photo-stimulated males. The photoperiodic treatment consisted in exposing the males to 2.5 months of long days (16 hours of light per day) from November 1st followed by exposure to natural photoperiodic conditions. Long days were provided with natural light and artificial light from 06:00 to 08:00 and from 18:00 to 22:00. Intensity of artificial light was at least 300 lx at the level of eyes of the animals. This photoperiodic treatment stimulates testosterone secretion, sexual behavior and vocalizations of bucks in March and April, during the natural sexual rest when control males are sexually inactive [11,22].

2.4. Male effect

On April 3rd (day 0; 08:00 hours), the four groups of females were exposed to the photo-stimulated entire bucks (n = 1 male per group). The positive-control group remained in contact with males for 15 days (n = 12). The experimental groups were in contact with males for 1 (n = 15), 5 (n = 15) or 10 days (n = 14; Fig. 1). In groups of does exposed to male for 5, 10 and 15 days, male from each group was daily switched between groups to avoid a possible individual effect in the response of females to the presence of males due to different individual buck behavior and possible habituation of does. The distance between each group of females was more than 100 m, to prevent any risk of interference between groups due to the presence of the males [19]. To ensure that all photo-stimulated males were sexually active, their sexual behavior was observed for 15 min following their introduction in the groups of females: all males displayed nudging and anogenital sniffing when in contact with the anovulatory females (Fig. 2).

2.5. Data collection and sampling procedures

Most of goats exposed to males ovulate twice, 7-10 days after the first contact between both sexes. Therefore, a daily sample of blood to determine plasma progesterone concentrations for 17 days after the introduction of males, allows identifying the type of ovulatory response of females [4,9]. Thus, in the current study, 5-mL daily blood samples were obtained from day 0 to 16 after exposure to males to assess ovulatory activity by determining plasma concentrations of progesterone. Blood samples were collected by jugular venipuncture in tubes containing heparin. Plasma was obtained after centrifugation at 3500 x g for 30 min and stored at -20 ℃ until hormone concentrations were determined. Concentrations of plasma progesterone were measured by immunoenzymatic assay as previously described by Canépa et al. [31]. Sensitivity was 0.25 ng/mL. The intra- and inter-assay coefficients of variation were 4% and 7%, respectively. Females with progesterone concentrations ≥ 1.0 ng/mL were considered to that have ovulated [9]. In addition, ovulation rates were assessed by the number of corpora lutea observed in each female by transrectal ultrasonography with the same equipment described above, 6 and 15 days after introduction of the bucks [11].

2.6. Definitions and statistical analyses of data

Types of ovulatory cycle were determined using the duration of the luteal phase. According to duration of high plasma concentrations of progesterone (>1.0 ng/mL), we identified 2 types of luteal phases after introduction of males: short luteal phase (2-4 days), which corresponds with a short life-span corpora lutea and long luteal phase (> 5 days), which corresponds with a normal life-span corpora lutea [4,32]. Therefore, we identified 3 types of ovulatory response after introduction of males: i) females that displayed a short luteal phase followed by a new ovulation; these goats ovulated twice and we consider that they displayed a short ovulatory cycle; ii) females that displayed only a short luteal phase which was not followed by any increase in progesterone concentration; these goats ovulated once then entered in anovulation iii) females that displayed a normal luteal phase after the first ovulation; these goats ovulated once and we consider that they displayed a normal ovulatory cycle. Length of short ovulatory cycles was estimated as being the days between the first and second increase of progesterone above the threshold of 1.0 ng/mL from two consecutive ovulations. The total proportion of females that ovulated during the study, females that displayed short or long luteal phase, females that displayed short ovulatory cycle, and females with short luteal phase but showing no further ovulation were analyzed using the Chi-square test. When there was a statistical difference, the comparisons between groups were made using the Fisher's exact test. Considering that the Lillefors test revealed that the data of ovulation rate did not have a normal distribution, this variable was analyzed between groups with the Kruskal-Wallis test. The ovulation rate during the first and second male-induced ovulations within each group was compared using a Friedman two-way analysis of variance. The day of the first ovulation was estimated as being the day prior to the first increase of progesterone above the threshold of 1.0 ng/mL. Interval to first ovulation and duration of luteal phase were compared between groups using a one-way ANOVA. All statistical analyses were performed using the statistical package SYSTAT 13 [33]. Results are expressed as mean ± standard error of the mean.

3. Results

All goats exposed to sexually active males ovulated at least once during the experiment. Illustrative examples of patterns of plasma progesterone concentrations indicating ovulations are shown in Fig. 2. The proportion of females that ovulated and the ovulation rates at the first and second ovulations did not differ between groups regardless of the duration of contact between males and females ($P > 0.05$). In females exposed to male for 1 day, the percentage of goats that ovulated was greater in the first than in the second ovulation ($P < 0.05$). In contrast, in this same group, ovulation rate was greater in the second than in the first ovulation ($P < 0.05$; Table 1). In groups of does exposed to males for 5, 10 and 15 days, the percentage of females that ovulated and the ovulations rates did not differ between the first and second ovulations (Table 1). The interval between the introduction of males and the first ovulation, the percentage of goats that displayed luteal phases of normal or short duration, and the length of these short luteal phases did not differ between the four groups ($P > 0.05$; Table 2). However, the percentage of females that entered in anovulation after their first luteal phase of short duration was higher in goats exposed to males for 1 day than in those exposed to males for 10 or 15 days ($P < 0.05$). This percentage is intermediate in the females in contact with males for 5 days, which did not differ from the 3 other groups ($P>0.05$; Table 2).

4. Discussion

Our results show that 1-day of contact with photo-stimulated bucks is long enough to induce ovulations in anovulatory goats. In fact, the proportion of goats that ovulated was not reduced by the time of contact with males, and all females exposed to males for 1, 5, 10 and 15 days ovulated. In addition, these data show that the percentage of does displaying a short luteal phase followed by anovulation was higher in females exposed to males for 1 day than in those remaining in contact with males for 10 or 15 days. Interestingly, the other characteristics of the ovulatory response such as the percentages of does that displayed short or normal luteal phase, or short ovulatory cycles and the ovulation rates did not differ between groups. Taken together, our results showed for the first time, that 1-day of contact with photo-stimulated and therefore sexually active bucks are sufficient to induce the ovulations in seasonally anestrous goats.

In the current study, most of the characteristics of the ovulatory response of goats from the four groups did not differ. The patterns of the ovulatory response registered in our study coincide with those reported previously in goats exposed continuously (24 hours per day) or intermittently (4, 2, or 1 hours per day) to photo-stimulated bucks for 15 consecutive days [6,19,20,29]. In contrast, our results did not agree with those reported in ewes, in which the reduction of time of contact between both sexes decreased the percentage of ewes that ovulated: 61% of ewes ovulated when remained in contact with wethers treated with testosterone for 15 days, but these percentages decreased to 53% and 18% when females were in contact with males for 4 and 1 day, respectively [17]. The origin of the difference between the results reported in ewes and ours is unknown. In our study, males were exposed to long days followed by natural photoperiod, a photoperiodic treatment that stimulates the endocrine and sexual activities of bucks during the non-breeding season [11,19,20,21]. These photo-stimulated males displayed intense sexual activity when in contact with females, as reported previously [5,11,21,29]. In our study, the high proportions of goats that ovulated in the control and experimental groups could be related to the use of the photo-stimulated males, and various studies agree with this hypothesis [5,11,20,21]. Indeed, male goats rendered sexually active by exposure to long days, induced ovulation in most females (>90%), whereas a low proportion did so when exposed to males in sexual rest, which displayed a weak sexual behavior [11,12]. Interestingly, the photo-stimulated bucks displaying an intense sexual behavior increased and maintained high plasma concentrations of LH in goats, leading to ovulation. By contrast, sedated photo-stimulated males that did not display any sexual behavior, but a strong odor, as awaked bucks, induced only a short-term LH activation and failed to stimulate ovulation [6,7,8]. Our results and those described above, strongly suggest that the sexual behavior displayed by the photo-stimulated males is an important factor determining the response of females exposed to them, and we can hypothesizes that it may have participated to induce the high ovulatory response observed in our study.

The proportion of goats that displayed a short luteal phase (=short life-span corpora lutea) followed by anovulation was higher in does exposed to males for 1 day, than in those that remained with males for 10 or 15 days. This pattern of ovulatory activity is consistent with previous results reported in sheep, in which all responsive ewes in contact with rams for 1 day (18%), displayed a short life-span corpora lutea followed by anovulation; furthermore, the proportion of females that displayed short life-span corpora lutea followed by anovulation was higher in ewes exposed to rams for 4 days (50%), than in those that remained in contact with them for 15 days [22%; 17]. Our results and those reported in ewes strongly suggest that the presence of males is crucial to avoid the females enter in anovulation after the first ovulation characterized by a short luteal phase.

In most females exposed to males for 1, 5, 10 or 15 days, the first elevation (≥ 1.0 ng/mL) of plasma progesterone concentrations occurred between 3 and 6 days, and the second one, between 9 and 12 days after exposure to males. Given that the increase of progesterone occurs 2–4 days after ovulation [24], our results suggest that the first and second induced-ovulations induced in does in contact with males for 10 and 15 days occurred in presence of males. In contrast, the first and second ovulations in does exposed to bucks for 1 day, and the second ovulation of those exposed to males for 5 days, occurred without the presence of males. The mechanism responsible for the ovulatory response of females that ovulated in absence of males is unknown. In sheep, it was suggested that the continuous presence of rams might be involved in maintaining a functional corpus luteum or in stimulating the formation of a new corpora lutea determining the duration of the ovulatory cycles [17]. In the current study, the ovulatory response of most of goats in contact with males for 1 or 5 days can be explained by the following arguments. Firstly, in our study, the relationships between females could enhance the ovulatory response in females from these two groups. In fact, it was shown that females in estrus could stimulate the estrous behavior and ovulation in other females by means of a “female effect” [34,35]. This could explain the first and second ovulations in most does exposed to males for 1 or 5 days, respectively. Secondly, it is possible that once the hypothalamo-pituitary-gonadal axis is stimulated by the presence of sexually active bucks for 1 day, secretion of GnRH and LH allows most of does to ovulate twice. This hypothesis does not agree with the fact that secretion of LH decreased once males are removed from females, thus preventing ovulation [6,17]. However, in goats it was recently shown that a daily contact between both sexes for 2 or 24 hours induced ovulations in both groups despite a different pattern of secretion of LH [6]. Together, the current results strongly suggest that the continuous contact between does and bucks is not necessary to induce ovulations in seasonally anestrous goats when exposed to photo-stimulated, sexually active bucks.

5. Conclusions

We conclude that 1 day of contact with photo-stimulated, sexually active bucks, is enough to induce the ovulatory activity in seasonally anovulatory goats. However, in this situation, a significant proportion of females do not ovulate again after displaying a short luteal phase.

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Competing interests

None of the authors have any conflict of interest to declare.

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Figures Legends

Fig. 1. Experimental design showing the duration of presence of bucks rendered sexually active by exposure to long days (16 hours of light per day) from November 1st to January 15th among the four groups of goats. Goats were exposed to the sexually active bucks for 1, 5, 10 or 15 consecutive days. The arrow indicates the introduction of males within the groups of females.

Fig. 2. Individual total number of occurrences of nudging and anogenital sniffing of bucks rendered sexually active by exposure to long days (16 hours of light per day) from November 1st to January 15th. The sexual behavior of males was observed for 15 min following their introduction in the groups of females. Males remained in contact with females for 1 (◩), 5 (■), 10 (□) or 15 (⊟) consecutive days.

Fig. 3. Illustrative examples of patterns of plasma progesterone concentrations in goats exposed to bucks rendered sexually active by exposure to long days (16 hours of light per day) from November 1st to January 15th. Goats were exposed to sexually active bucks for 1 (●), 5 (○), 10 (■) or 15 (□) consecutive days. Males were introduced into the groups of goats on day 0 and their presence is indicated by horizontal bars within each group.

Table 1. Body condition score (BCS) and ovulatory response of seasonally anestrous goats exposed to sexually active bucks for 1, 5, 10 or 15 consecutive days. Bucks were rendered sexually active by exposure to long days (16 h of light per day) from November 1st to January 15th followed by natural photoperiodic conditions.

Groups	n	[1]BCS (Mean±SEM)	First ovulation		Second ovulation	
			Females with ovulations (%)	Ovulation rates (Mean±SEM)	Females with ovulations (%)	Ovulation rates (Mean±SEM)
1 day	15	2.1 ± 0.10	15/15 (100) ^{a}	1.3 ± 0.13 ^{x}	7/15 (47) ^{b}	1.9 ± 0.14 ^{y}
5 days	15	2.2 ± 0.08	15/15 (100)	1.3 ± 0.13	10/15 (67)	1.6 ± 0.15
10 days	14	2.0 ± 0.08	14/14 (100)	1.5 ± 0.14	10/14 (71)	1.6 ± 0.15
15 days	12	2.0 ± 0.14	12/12 (100)	1.4 ± 0.15	8/12 (67)	1.7 ± 0.17
		NS	NS	NS	NS	NS

[1] BCS = Body Condition Score. Range: from 1 (very thin) to 4 (very fat).
Differences among groups were not significant (NS)
$^{a\text{-}b,\ x\text{-}y}$Different superscripts within each row donote significant difference (*P*>0.05).

Table 2. Characteristics of the duration of the luteal phase in seasonally anestrous goats exposed to sexually active bucks for 1, 5, 10 or 15 consecutive days. Bucks were rendered sexually active by exposure to long days (16 h of light per day) from November 1st to January 15th followed by natural photoperiodic conditions.

Groups	n	Interval to first ovulation (days)	Goats with long luteal phase (%)	Goats with short luteal phase followed by a new ovulation (%)	Length of short ovulatory cycles (days)	Goats with short luteal phase followed by anovulation (%)
1 day	15	3.2 ± 0.18	3/15 (20)	7/15 (47)	6.7 ± 0.52	5/15 (33) ^{a}
5 days	15	3.5 ± 0.30	2/15 (13)	10/15 (67)	6.9 ± 0.23	3/15 (20) ^{a,b}
10 days	14	3.8 ± 0.21	4/14 (29)	10/14 (71)	7.1 ± 0.21	0/14 (0) ^{b}
15 days	12	3.2 ± 0.18	4/12 (33)	8/12 (67)	7.2 ± 0.22	0/12 (0) ^{b}

^{a,b}Different superscripts within each column donote significant difference (*P*<0.05).

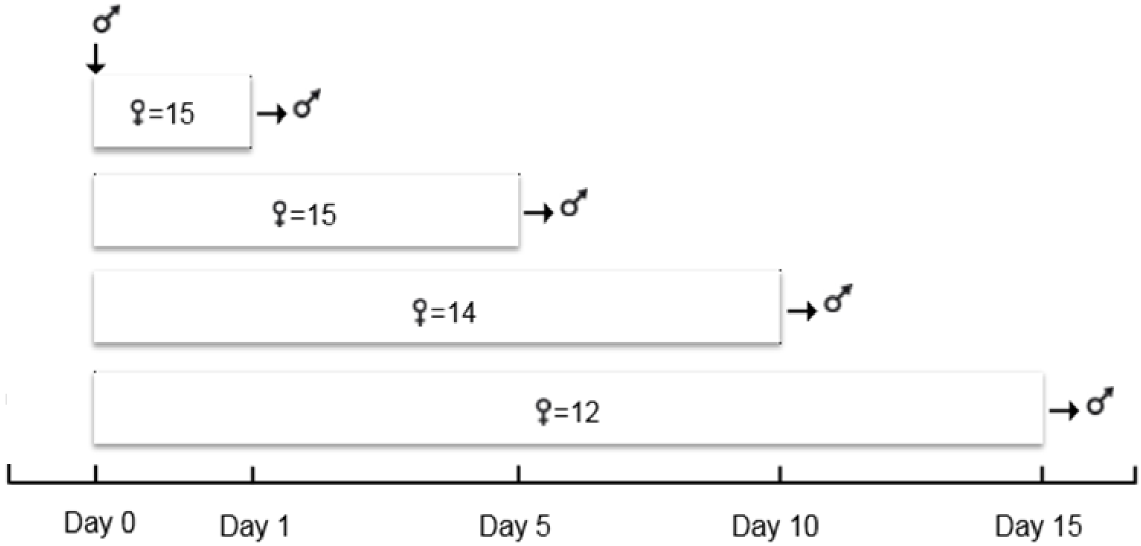


Fig. 1.

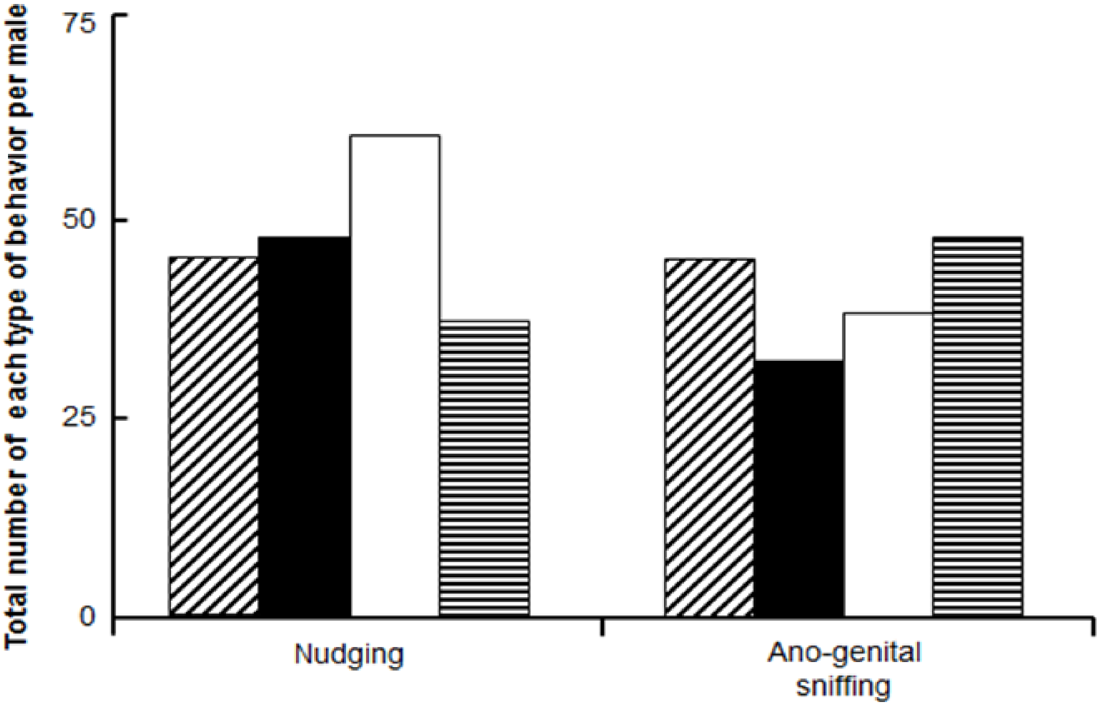
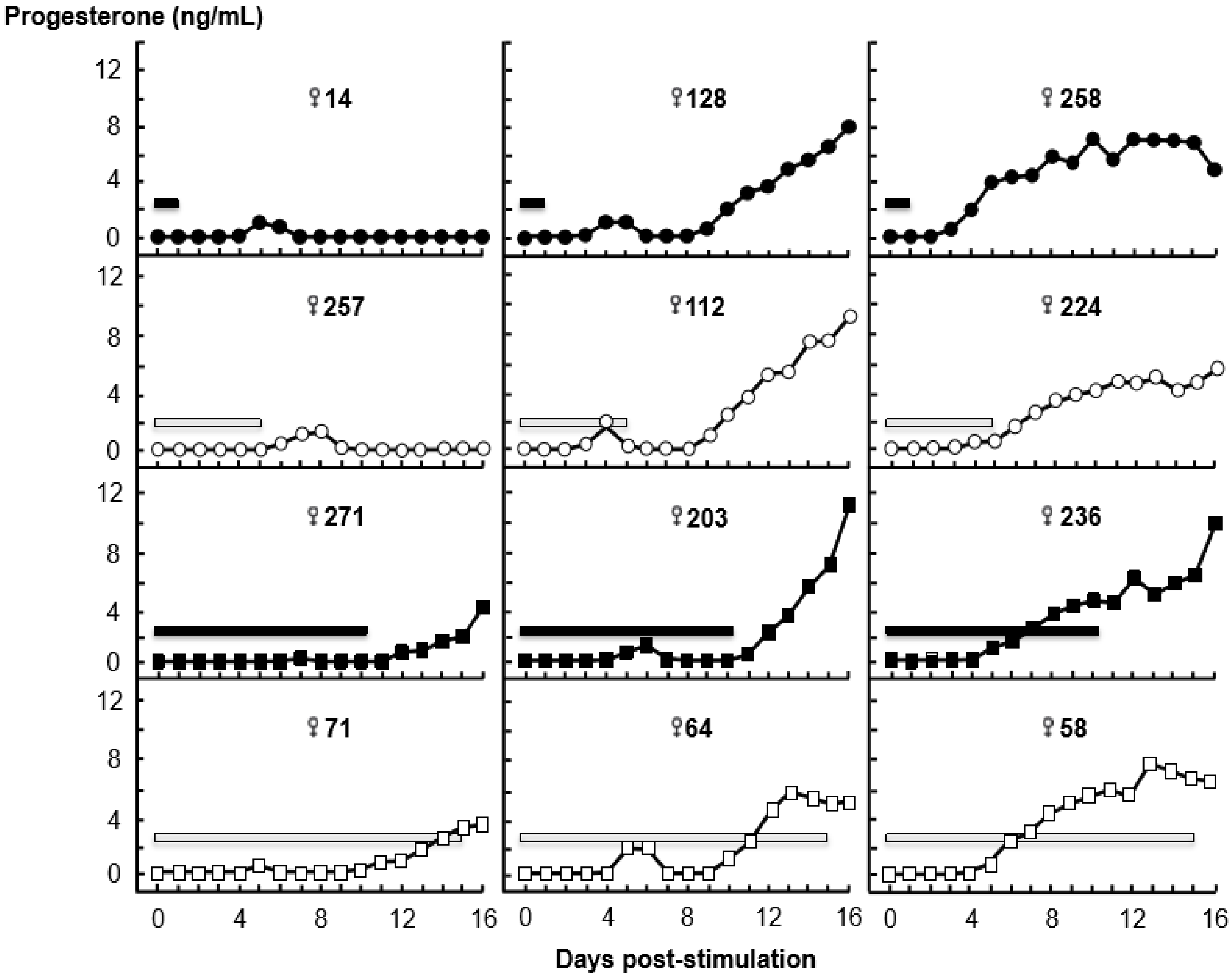

Total number of each type of behavior per male
75
50
25
0
Nudging
Ano-genital sniffing

Fig. 2.



Progesterone (ng/mL)
12
8
4
0
♀14
♀128
♀258
♀257
♀112
♀224
♀271
♀203
♀236
♀71
♀64
♀58
0
4
8
12
16
Days post-stimulation

Fig. 3

- Fifteen days of contact with photo-stimulated bucks induce ovulation in goats
- Also, one day of contact with photo-stimulated bucks induce ovulation in goats
- However, a third of goats fall again in anovulation after a short luteal phase

VII. CONCLUSIONES Y PERSPECTIVAS

Los resultados del primer estudio demuestran que acortar el tratamiento fotoperiódico de 75 a 15 DL es suficiente para inducir una intensa actividad sexual de los machos, determinada por la secreción de testosterona durante el periodo de reposo sexual. Además, la reducción 75 a 30 DL, habilita a los machos para estimular la actividad ovulatoria de las cabras anéstricas mediante el efecto macho. Este hallazgo podría resultar interesante para mejorar los sistemas productivos al simplificar el tratamiento fotoperiódico y posiblemente reducir los gastos generados por energía eléctrica y alimentación para la inducción de la actividad sexual de los machos. Sería interesante investigar si los machos tratados con tan solo 15 DL son capaces de inducir la actividad ovulatoria y alcanzar en las hembras porcentajes de gestación comercialmente aceptables.

Los resultados del segundo estudio indican que la reducción del tiempo de contacto entre machos y hembras de 15 días hasta 1 día, no modifica la habilidad de los machos foto-estimulados para inducir la actividad ovulatoria de las cabras anéstricas. Sin embargo, el hallazgo es importante porque permite conocer el alcance fisiológico de las hembras, para responder a la bioestimulación sexual.

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IX. ANEXOS

Resumen 1

P166.- Reducing duration of long-day treatments does not modify the capacity of male goats to induce ovulations in anestrous does.

Presentado: In Proceedings of the 17th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), Bologna Italy. Vol. 48. Supplement 1, September 12–14th, 2013, 1–124.

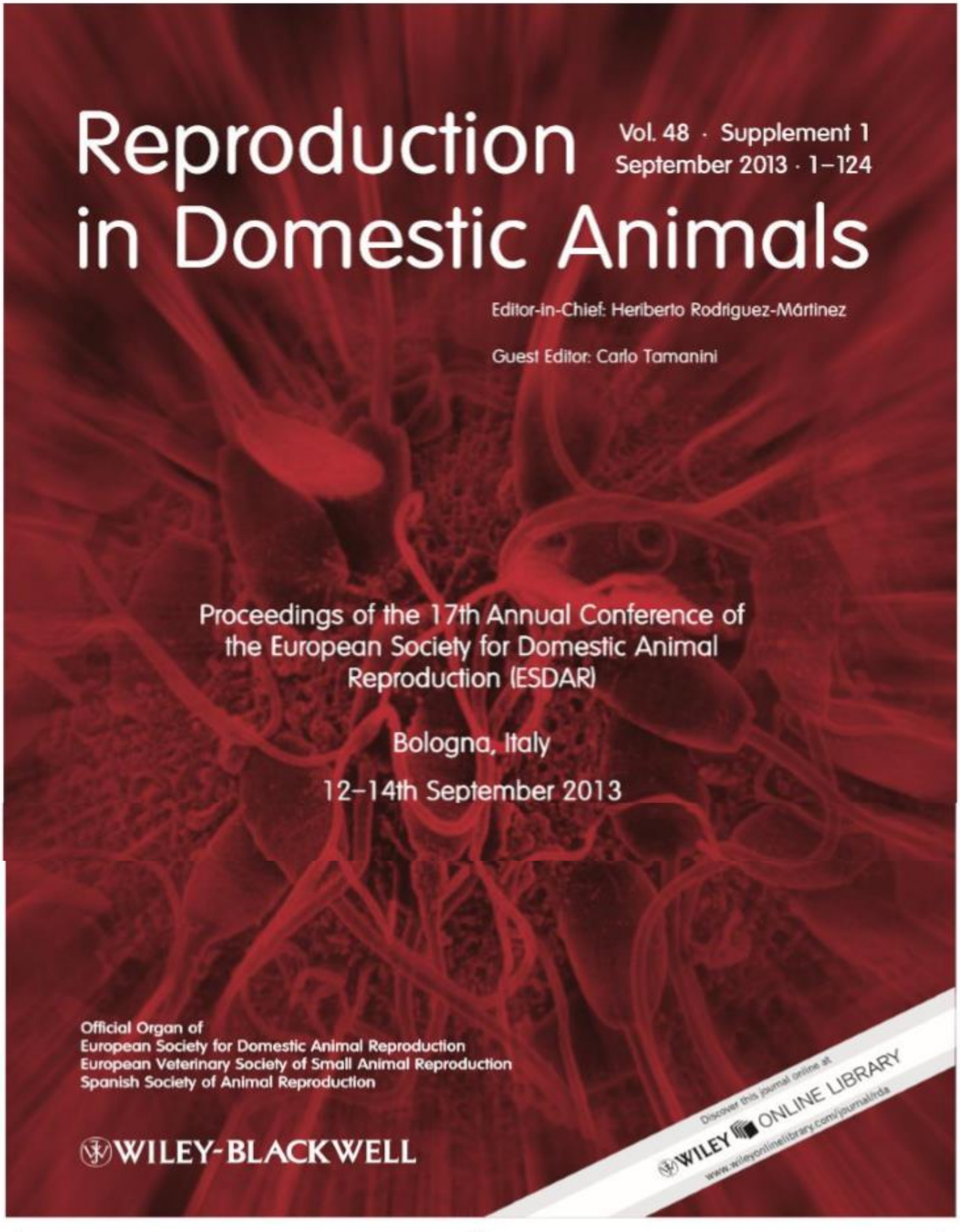
Resumen 2

P149.- One day of contact with the photo-stimulated bucks is sufficient to induce ovulation in seasonally anovulatory goats.

Presentado: In Proceedings of the 18th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), and the Annual Meeting of EU-AI-Vets, Helsinki Finland. Vol. 49. Supplement 3, September 11–13th, 2014, 37–101.

Resumen 1

P166.- Reducing duration of long-day treatments does not modify the capacity of male goats to induce ovulations in anestrous does.



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Local signs of inflammation regressed after 4 days and the mare was discharged. The mare resumed work after 45 days and signs of discomfort rapidly regressed. Reproductive prognosis will depend on the cervix functionality that hasn't yet been assessed. L vs. can be a primary or secondary cause of infertility, increase the risk of dystocia or alter sport performance. However, it is often asymptomatic and an incidental finding. Consequently, it is most likely under-diagnosed which accounts for the very few reports of this condition in mares.

P165

Comparison of the effect of dexamethasone and flunixin meglumine administered to mares at insemination time in the modulation of mating induced endometritis

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Recently, successful treatment of mating induced endometritis with a single dose of dexamethasone or with non-steroidal anti-inflammatory drugs administered at the time of breeding has been reported in mares. However, a higher incidence of ovulatory failure in mares treated with high doses of flunixin meglumine has been reported. This study compares the anti-inflammatory effects of dexamethasone or flunixin meglumine administered at the time of insemination. Thirty three Standardbred mares inseminated with cooled semen were included. Intrauterine fluid, uterine folds, and number and dimension of follicles or corpora lutea were recorded at ovulation induction time (administration of 2000 UI human chorionic gonadotropin; V1) at insemination time (V2) and 24 h after insemination (V3). At V2 11 mares received a single dose of dexamethasone (50 mg) (D group), 11 flunixin meglumine (500 mg F group) and 11 mares saline (10 ml, C group). Uterine cytology samples were collected by brush at V1 and V3. The number of neutrophils in uterine cytology was significantly increased in V3 compared to V1 only in the C group ($p = 0.044$). Intrauterine fluid observation, occurrence of ovulation failure and corpora lutea diameter did not differ among the three groups. A significant reduction of uterine folds was observed from V1 to V3 in the D group ($p < 0.0001$) and in the C group ($p = 0.0002$). In conclusion, the administration of the tested anti-inflammatory drugs reduces the number of neutrophils in endometrial cytology samples and does not affect the incidence of ovulation failure.

P166

Reducing duration of long-day treatments does not modify the capacity of male goats to induce ovulation in anestrous does

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The objective of the study was to determine whether male goats rendered sexual active by exposure to different long-days (LD) treatments are able to induce ovulation in seasonal anestrous goats. Males were allocated to three groups and kept in open pens ($n = 5$ each). One group was submitted to 75 days of LD (16 h light/day) from November 1st (75-LD); the second group was subjected to 45 days of LD from December 1st (45-LD); the third group was subjected to 30 days of LD from December 15th (30-LD). All treatments ended on January 15th; then, males were kept under natural photoperiodic variations. Multiparous female goats were used. One group was isolated from males ($n = 13$). On April 2, 3 experimental groups of females were exposed during 15 days to one of the three male groups ($n = 3$ males per group; 75-LD: $n = 25$; 45-LD: $n = 27$, and 30-LD: $n = 26$). Ovulations were assessed by transrectal ultrasonography 17 days after being exposed to bucks. The proportions of goats that ovulated were greater in those in contact with males from 75-LD (92%), 45-LD (89%) or 30-LD (88%) than in those isolated from them (0%; $p < 0.05$). There were not differences in the proportions of females that ovulated when exposed to the treated males ($p > 0.05$). These results indicate that reduction of long-day treatments from 75 to 30 days does not modify the capacity of male goats to stimulate the ovulatory activity in goats during seasonal anestrous.

P167

Preliminary results: early sex determination in the canine foetus by echotomography

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Foetal sex determination is a key element in the economics of animal breeding; the value and management of progeny is highly dependent on gender. Twenty-five bitches were monitored between 25 and 50 days of gestation to determine the sex of their foetuses by ultrasonography using a 12.5 MHz probe. After the 31st day, sexing was possible due to the position of the genital tubercle in relation to the pelvic limbs, tail, and umbilical cord. On ultrasound 34 females and 36 males were detected on day 33, at birth there were 30 females and 40 males (including the 10 foetuses autopsied at 35 days). The external genital organs of 10 foetuses autopsied after caesarean section at 35 days of gestation, were examined macroscopically and histologically. A novel PCR technique was developed to detect the SRY gene in formaldehyde-preserved tissues taken from the 10 foetuses. Histologically, cartilaginous differentiation was visible in both the male (penile buds) and female (possibly relating to clitoral tissue). PCR testing of the autopsied foetuses gave accurate results with eight females and two males. There was good statistical correlation between sexing by ultrasonography and sexing at birth (Kappa coefficient 0.8, percentage error 8.5%). Prenatal sexing is possible from day 31 of gestation in bitches.

P168

Pre-pubertal female goats in socio-sexual contact with males advanced the onset of puberty

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Puberty is the age at which a female goat has her first ovulation and/or oestrous behaviour. The aim of this study was to determine whether the presence of bucks modify the onset of puberty. Female kids were born in January (17 ± 2 days) and separated from their mother at 3 days of age. All females were fed with goat milk until weaning at day 40. Thereafter, females were randomly assigned to one of two groups ($n = 9$ each). One group of goats remained isolated from males and the other group had visual, olfactory, auditory and restricted tactile contact with males through a fence. Blood samples were taken at weekly intervals to assess plasma progesterone concentrations. Values > 1 ng/ml were considered indicative of puberty. The proportions of does that ovulated were analyzed by Chi square test. Proportions of does that reached puberty at 297 days of age was different between does in contact with males (9/9; 100%) than isolated ones (5/9; 55%; $p = 0.02$). In those does, puberty was reached at 269 ± 4.7 days of age. We conclude that female goats in socio-sexual contact with males had advanced onset of puberty.

Resumen 2

P149.- One day of contact with the photo-stimulated bucks is sufficient to induce ovulation in seasonally anovulatory goats.

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tract and, consequently, of their reduced fertilization efficiency in comparison with LS-sperm when used in AI programs. (Supported by MINECO (AGL2012-399903), Madrid, Spain and FEDER Funds).

P148

L. vaginalis, *B. pumilus* and *T. pyogenes* have different effects on the expression of interleukins in bovine endometrial epithelial cells *in vitro*

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After parturition, a bacterial invasion of the bovine uterus is common and can result in inflammation of the endometrium and reduced reproductive performance. The aim was to compare the effects of *Lactobacillus vaginalis*, *Bacillus pumilus* and *Trueperella pyogenes* on the expression of different interleukins (IL1α, IL6 and IL8) in bovine endometrial epithelial cells. Cells were co-cultured with the bacteria in different multiplicities of infection (MOI; *L. vaginalis* and *B. pumilus*: MOI = 1, 5, 10; *T. pyogenes*: MOI = 0.1, 0.5, 1). All MOI of *L. vaginalis* did not have an effect on the cell viability for up to 96 h. In contrast, *B. pumilus* and *T. pyogenes* caused death in >95% of the epithelial cells within 24 h and 16 h, respectively. After 2 h, 4 h and 6 h, total RNA was extracted and subjected to real-time RT-PCR. Compared with untreated controls, the co-culture with *L. vaginalis* did not result in an increased interleukin expression, whereas cells co-cultured with *B. pumilus* showed for all MOI a significantly higher expression of all factors at each point of time, with the highest expression after 2 h. Co-culture with *T. pyogenes* showed an increased expression of IL6 after 6 h with MOI = 0.5 and 1. The expression of IL8 increased after 2 h with all MOI, but also with the highest expression after 6 h and MOI = 1. These results suggest that epithelial cells respond to pathogenic bacteria with a higher synthesis of interleukins, but are also more likely to be killed by these bacteria. (Supported by DFG-GA1077/5-1).

P149

One day of contact with the photo-stimulated bucks is sufficient to induce ovulation in seasonally anovulatory goats

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The aim of the study was to determine the ovulatory response of anestrous goats exposed to the photo-stimulated bucks for 1 (n = 15), 5 (n = 15) or 15 (n = 12) days. Males were rendered sexually active during the sexual rest season by exposing them to 2.5 months of long days from November 1st. In April, during the seasonal anestrous, each group of goats was exposed to the photo-stimulated bucks (n = 1/group). In all goats, ovulations were determined by plasma progesterone levels. Proportions of females that ovulated, displayed short ovulatory cycles (<17 days) or developed short-livespan corpora lutea were compared using the χ^2 test. All goats from each group ovulated at least once during the experiment ($p > 0.05$). The proportions of goats that displayed short ovulatory cycles did not differ between females in contact with males for 1 (47%), 5 (67%) or 15 days (67%; $p > 0.05$). The proportions of females that ovulated once and developed short-livespan corpora lutea was higher in goats exposed to males for 1 day (33%) than in those exposed to males for 15 days (0%; $p < 0.05$). In contrast, these proportions did not differ between groups in contact with males for 5 (20%) or 15 days ($p > 0.05$). We concluded that 1 day of contact with photo-stimulated males is enough to induce ovulation in seasonally anovulatory goats.

P150

Decrease of stillbirth risk by integrated treatment in mares

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The purpose of this study was to investigate the impact of integrated treatment on the outcome of risk pregnancies. The study was performed on 30 mares in 3 groups on the 270–300th days of gestation. The Group 1 (control group) consisted of 10 healthy mares. For correction of proven metabolic disorders, 10 mares (Group 2) were treated with a combination of BF-15 and Heamobalans and 10 mares were treated with Haemobalans only (Group 3). The assessment of the results was based on the quality of the placental structure and vitality of newborn foals. Pieces of placenta were stained with routine hematoxylin and eosin. The criteria used for diagnosing placental condition were dystrophy, vasculature and quality of trophoblast cells. The combination of medications improved metabolism and prevented alimentary abortion. The placental structure in the Group 2 approximated to the structure in the Group 1. The positive influence of integrated treatment on the outcome was proven by an increase in the number of functioning chorionic villi (this area is larger ($p \le 0.05$) at 8.3% in Group 2 than in Group 3, but is less ($p \le 0.05$) at 1.3% than in the Group 1). There were no cases of stillbirths in the Group 2 as well as in the Group 1, but two cases in the Group 3. The number of mares with diarrhea did not differ significantly between the groups. The integrated treatment of Heamobalans and BF-15 improved the outcome of pregnancy in mares in risk. It can be recommended as the preventive treatment in cases of placental and metabolic insufficiency.

P151

Does the presence of *Enterobacter cloacae* in boar semen negatively affect sperm preservation at 15–17°C?

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The contamination of fresh and extended boar semen often occurs in farms and artificial insemination centres during semen collection, processing and storage. Apart from its negative impact on sows, contamination of semen could cause detrimental effects on semen quality and may compromise its storage in liquid state at 15–17°C, as reported for *Escherichia coli* and *Clostridium perfringens*. Since no previous study evaluated how *Enterobacter cloacae* affects the preservation of boar sperm in liquid storage at 15–17°C, the present work sought to determine how this bacterium affected motility and membrane integrity of boar spermatozoa during an 11-day period. With this purpose, five extended semen samples from five healthy post-pubertal boars were artificially contaminated with *Enterobacter cloacae* in five different sperm:bacteria ratios (2 : 1; 1 : 1; 1 : 5 and 1 : 10), the 1 : 0 ratio (non-inoculated) being the negative control. Sperm motility was evaluated through computer assisted sperm analysis (CASA), whereas sperm membrane integrity was evaluated by SYBR14/PI staining and flow cytometry. Results showed that the highest bacterial concentrations (i.e. 1 : 5 and 1 : 10) had a significantly ($p < 0.05$) higher damaging-effect on sperm motility and membrane integrity than the lowest one (2 : 1) and negative control (1 : 0). In conclusion, this work demonstrates that the presence of *Enterobacter cloacae* in boar semen doses also compromises sperm