UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

LA INTRODUCCIÓN DE MACHOS CABRÍOS NUEVOS AL DÍA 23 DE INICIADO EL EFECTO MACHO NO AUMENTA LA TASA DE GESTACIÓN AL DÍA 55

Artículo

Que presenta JHONNATAN ARAYA SALAS

como requisito parcial para obtener el Grado de

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ÍNDICE GENERAL

I. Introducción

En algunas razas de ovejas y cabras que muestran estacionalidad reproductiva, la introducción de un macho en un grupo de hembras en anestro estacional puede inducir su actividad sexual dentro de los primeros 5 días de contacto entre ambos sexos. En ovejas y cabras, este fenómeno de bioestimulación sexual se conoce como el "efecto macho". Varios factores modifican la respuesta de las hembras al efecto macho, incluyendo la novedad del macho y la intensidad del comportamiento sexual desplegado por los machos. En los ovinos, la proporción de hembras que ovulan es mayor cuando se exponen a machos "nuevos" (machos desconocidos para las hembras) que a machos familiares (machos conocidos para las hembras; Hawken et al., 2009; Jorre de St Jorre et al., 2012). En caprinos, la proporción de hembras que ovula es más elevada cuando se exponen a machos inducidos a una intensa actividad sexual durante el periodo de reposo al exponerlos a días largos en otoño e invierno (machos fotoestimulados), que a machos control que despliegan débil comportamiento sexual (Delgadillo et al., 2006; Flores *et al.,* 2000).

La tasa de gestación promedio registrada en cabras expuestas a machos fotoestimulados durante 15 días, es del 75% (rango del 58% al 81%; Bedos et al, 2010; Fitz-Rodríguez et al., 2009). Parte de las hembras que no quedan gestantes pueden mostrar una fase lútea de duración normal, y luego entrar en anovulación, incluso cuando los machos permanecen con las hembras durante 35 días consecutivos (Delgadillo-Sánchez et al., 2003; Flores et al., 2000). Otras cabras ovulan y muestran un comportamiento estral entre 24 y 28 días después del primer contacto con los machos (Delgadillo-Sánchez et al., 2003; Flores et al., 2000; Ott et al., 1980).

La variabilidad de la tasa de gestación registrada con la presencia de los machos durante 15 días, sugiere que existe un margen para mejorar la tasa de gestación. Considerando el efecto positivo de la novedad de los machos sobre la ovulación en ovejas, y la eficacia de los machos fotoestimulados para inducir la ovulación en cabras, el objetivo del presente estudio fue determinar si la introducción de machos "nuevos fotoestimulados" el día 23 después del primer contacto con otros machos, incrementa la tasa de gestación cuando las hembras y machos permanecen en contacto durante 35 días.

II. Objetivo

El objetivo de este estudio fue determinar si el cambio de machos cabríos por nuevos machos fotoestimulados el día 23 después de la introducción de los machos mejora la tasa de gestación en las cabras expuestas durante 35 días a machos sexualmente activos.

III. Hipótesis

El cambio de machos caprinos por nuevos el día 23 después del primer contacto, es decir, después de la segunda ovulación, mejora la tasa de gestación en un grupo de cabras cuando se realiza un efecto macho durante 35 días, debido a un efecto de la novedad de los machos.

IV. Revisión de literatura

4.1 Estacionalidad reproductiva de los mamíferos

La estacionalidad de la reproducción, como parte del proceso de selección natural, es un mecanismo de adaptación desarrollado por algunos mamíferos silvestres como estrategia para minimizar el impacto negativo del ambiente (temperatura, humedad y disponibilidad de alimento) sobre la supervivencia de las crías (Karsch et al., 1984; Malpaux et al., 1996). Por ello, en las especies que manifiestan estacionalidad reproductiva, los nacimientos de las crías ocurren en la época más favorable del año, con abundancia de pastos y temperatura ambiental confortable.

4.2 Estacionalidad reproductiva en caprinos

La estacionalidad reproductiva es una característica de algunas razas caprinas originadas o adaptadas a las regiones subtropicales (Delgadillo, 2011; Restall, 1992; Rivera et al., 2003; Walkden-Brown et al., 1994). En las hembras caprinas no gestantes, el anestro estacional se caracteriza por la ausencia de estros y ovulaciones. Por el contrario, la estación sexual se caracteriza por la sucesión, cada 21 días, de ciclos estrales y ováricos. Así, en las hembras de la raza Cashmere de Australia, en el hemisferio sur (29º S), la actividad sexual ocurre de febrero a agosto (otoño-invierno), y el periodo de anestro estacional o reposo sexual, ocurre de septiembre a enero (primavera-verano; Restall, 1992). De manera similar, en las hembras caprinas locales de la Comarca Lagunera, ubicada en el norte de México, en el hemisferio norte (26º N), el periodo de actividad sexual ocurre de septiembre a febrero (otoño-invierno), y el anestro estacional ocurre de marzo a agosto (primavera-verano; Duarte *et al.,* 2008).

Los machos caprinos de las razas antes mencionadas manifiestan también variaciones estacionales de su actividad sexual. Así, durante el periodo de reposo sexual, la secreción de testosterona, el comportamiento sexual, el olor, y la producción espermática disminuyen considerablemente. En cambio, estas variables aumentan durante la estación sexual (Delgadillo et al., 1999; Rivas-Muñoz et al., 2007; Walkden-Brown et al., 1994). En los machos cabríos de la raza cashemere de Australia, la estación sexual inicia en noviembre y termina en mayo (verano-otoño), y el periodo de reposo sexual inicia en junio y termina en octubre (invierno-primavera; Walkden. Brown et al., 1994). De manera similar, en los machos locales del norte de México, la estación sexual ocurre de junio a diciembre (verano-otoño), y el reposo sexual de enero a mayo (invierno-primavera; Delgadillo et al., 1999).

4.3 Fotoperiodo y estacionalidad sexual de los caprinos

El fotoperiodo es el principal factor del medio ambiente responsable de la estacionalidad reproductiva de los caprinos de latitudes subtropicales, al igual que en los caprinos de latitudes templadas (Delgadillo y Chemineau, 1992; Delgadillo et al., 2004; Duarte et al., 2010). El efecto del fotoperiodo sobre la reproducción de los caprinos de latitudes subtropicales se determinó cuando machos y hembras se alojaron por separado en edificios cerrados, y se sometieron a 3 meses de días largos artificiales (14 h de luz por día), seguidos de 3 meses de días cortos artificiales (10 horas de luz por día) durante 2 años consecutivos. En estas condiciones experimentales, la secreción de testosterona y las ovulaciones se estimularon siempre durante los días cortos y se inhibieron durante los días largos (Delgadillo et al., 2004; Duarte et al., 2010). Estos datos sugieren que en los caprinos de latitudes subtropicales, los días cortos estimulan la actividad sexual de machos y hembras, y que los días largos la inhiben. Sin embargo, en las hembras caprinas expuestas a las variaciones del fotoperiodo natural se demostró que el inicio de la estación sexual no se debe a la disminución del fotoperiodo, sino a la aparición del estado refractario a los días largos (Delgadillo et al., 2011). En efecto, el inicio de la estación sexual determinada por las concentraciones plasmáticas de LH en hembras ovariectomizadas a las que se les coloco un implante subcutáneo que libera constantemente estradiol (OVX+E), no fue diferente entre las hembras en fotoperiodo natural y en las mantenidas en días largos desde el solsticio de invierno (Delgadillo et al., 2011). En ambos grupos, las concentraciones plasmáticas de LH se incrementaron en septiembre, mes que corresponde al inicio de las ovulaciones en las hembras intactas (Delgadillo et al., 2011; Duarte et al., 2008). De manera similar, el final de la estación sexual de las cabras de latitudes subtropicales no se debe al incremento en la duración del fotoperiodo, sino a la aparición del estado refractario a los días cortos. Así, en las hembras OVX+E, el final de la estación sexual no fue diferente entre las hembras en fotopertiodo natural y en aquellas mantenidas en días cortos desde el solsticio de invierno (Delgadillo et al., 2011). En ambos grupos, las concentraciones plasmáticas de LH disminuyeron en febrero, mes que corresponde al final de las ovulaciones en las hembras intactas (Delgadillo et al., 2011; Duarte et al., 2008). Estos últimos datos sugieren que en los caprinos de latitudes subtropicales, la duración del día no estimula o inhiben la actividad sexual en las hembras, sino que sincroniza, muy probablemente, el ritmo endógeno de reproducción, tal y como ocurre en caprinos y ovinos de latitudes templadas (Gebbie et al., 1999; Karsch et al., 1989; Gómez-Brunet et al., 2010). En los machos cabríos de latitudes subtropicales, es probable que el fotoperiodo tenga el mismo papel que en las hembras, y que sincronice también el ritmo endógeno de reproducción, tal y como ocurre en los machos originarios de latitudes templadas (Howles et al., 1982; Lincoln y Almeida, 1984). En conjunto, los resultados descritos anteriormente sugieren que la modificación artificial del fotoperiodo, es decir, la aplicación de días artificiales largos y/o cortos, permite modificar el ritmo anual de reproducción de machos y hembras, induciendo su actividad sexual durante los periodos de reposo sexual (Delgadillo et al., 2001; Delgadillo et al., 2015; Flores et al., 2000).

4.4 Tratamientos fotoperiódicos para estimular la actividad sexual de los caprinos

La modificación artificial del fotoperiodo permite que los animales que presentan estacionalidad reproductiva, manifiesten actividad sexual durante los meses de reposo sexual, permitiendo su reproducción (Chemineau et al., 1992; Delgadillo, 2011; Flores et al., 2000; Ponce et al., 2014). Para estimular la actividad sexual de los caprinos, los animales deben percibir alternancias entre días largos y días cortos para evitar la aparición del estado refractario, es decir, la insensibilidad al fotoperiodo al que se encuentran sometidos en un momento determinado (Chemineau et al., 1992; Delgadillo et al., 2001). Los días largos se proporcionan con una iluminación complementaria a la luz natural, y los días cortos se simulan aplicando implantes de melatonina (hormona que otorga una señal de días cortos), días cortos artificiales, o el fotoperiodo corto natural (Delgadillo et al., 2014; Flores et al., 2000). En los machos cabríos de la Comarca Lagunera, su actividad sexual se estimula al someterlos a 2.5 meses de días largos (16 h de luz por día) del 1 de noviembre al 15 de enero, seguidos del fotoperiodo natural. Este tratamiento estimula la secreción de testosterona, el comportamiento sexual, el olor, y las vocalizaciones de los machos en marzo y abril, meses que corresponden al periodo de reposo sexual (Bedos *et al.,* 2012; Delgadillo *et al.,* 2002, 2012; Ponce et al., 2014; Vielma et al., 2009). En las hembras, las ovulaciones se inducen al someterlas a 2-3 meses de días largos, seguidos de melatonina o fotoperiodo natural. Este tratamiento induce la manifestación del estro y las ovulaciones durante el periodo de anestro estacional (Chemineau et al., 1992).

4.5 Uso del "efecto macho" para estimular la actividad sexual de las cabras durante el anestro estacional

En las hembras de razas caprinas que muestran estacionalidad reproductiva, su actividad sexual puede estimularse durante el anestro estacional al ponerlas en contacto con machos, lo cual se conoce como "efecto macho" (Delgadillo *et al.,* 2009; Flores *et al.,* 2000; Pellicer-Rubio *et al.,* 2008; Shelton, 1960; Walkden-Brown *et al.,* 1999).

4.6 Respuesta endocrina y sexual de las hembras caprinas expuestas a los machos

La respuesta inmediata de las hembras caprinas a la presencia de los machos es el incremento de la pulsatilidad de la LH (Bedos *et al.,* 2014; Chemineau, 1987; Hawken y Martin, 2012; Vielma *et al.,* 2008). La presencia continua del macho permite que se mantenga la secreción elevada de LH, facilitando la presentación del pico preovulatorio de LH para que ocurra la ovulación (Bedos *et al.,* 2014; Hawken y Martin, 2012; Martínez-Alfaro et al., 2014). La respuesta estral y ovulatoria de las cabras expuestas a los machos tiene una repetibilidad muy elevada. Las cabras anovulatorias expuestas a los machos ovulan en los primeros 5 días de contacto con ellos. Esta primera ovulación inducida por los machos se asocia al estro en una proporción variable de hembras. La mayoría de las cabras presentan un ciclo ovulatorio de corta duración, y 6 días después de la primera ovulacion ocurre la segunda ovulación que es acompañada, en la totalidad de las hembras, de un comportamiento estral. Si las cabras no son fecundadas en la segunda ovulación, éstas presentan una tercera ovulación acompañada de estro 21 días después. Además, las hembras que no hacen un ciclo estral de corta duración, depués de la primera ovulación, presentan un ciclo ovulatorio de duración normal, es decir, de 21 días, acompañado de estro (Chemineau, 1987; Chemineau et al, 2006; Ott et al, 1980; Walkden-Brown et al., 1999). Independientemente del perfil ovulatorio de las hembras expuestas a los machos (ciclos ovulatorios cortos o normales), de la presencia de machos intactos o vasectomizados, las cabras expuestas a los machos no mantienen su actividad ovulatoria de manera indefinida, aun cuando no están gestantes, y retornan al anestro estacional (Aroña, 2015; Chemineau, 1987; Delgadillo-Sánchez et al., 2003; Ott et al., 1980).

4.7 Factores que modifican la respuesta endócrina y sexual al efecto macho

En los pequeños rumiantes, como en los caprinos y ovinos, la respuesta al efecto macho puede ser modificada por varios factores incluyendo la novedad de los machos, así como la intensidad de su comportamiento sexual (Delgadillo et al., 2006; Flores et al., 2000; Hawken et al., 2009; Jorre de St Jorre et al., 2012; Rivas-Muñoz et al., 2007; Vielma et al., 2009).

a) Novedad de los machos

La respuesta ovulatoria de las ovejas no es la misma si son expuestas a machos “familiares” (machos conocidos de las hembras) o “nuevos” (machos desconocidos de las hembras (Hawken et al., 2009; Jorre de St Jorre et al., 2012). En efecto, después de 3 meses de contacto con machos ovinos, éstos se retiraron por 15 min de las ovejas. A los 15 min de haberlos retirado, las ovejas se re-expusieron a machos familiares, con los que habían estado en contacto por 3 meses, o machos nuevos y desconocidos por las hembras. La secreción de LH se incrementó solamente en las hembras que se expusieron a los machos nuevos (Hawken et al., 2009). De manera similar, en otro estudio se demostró que la mayoría de las ovejas ovulan cuando se exponen a machos nuevos, mientras que solo un tercio de ellas ovula al ponerlas en contacto con machos familiares (Jorre de St Jorre et al., 2012). Al momento de realizarse el presente estudio no había disponible, a nuestro conocimiento, datos que indiquen si la novedad de los machos caprinos incrementa la respuesta endocrina y sexual en las cabras al ser expuestas a los machos. En conjunto, estos resultados sugieren que en ovinos, la novedad del macho es un factor importante para reactivar el funcionamiento del eje hipotálamo-hipófisis-gónadas en las hembras anéstricas expuestas a los machos.

b) Comportamiento sexual de los machos

Otra variable que puede modificar la respuesta endocrina y sexual de las cabras y ovejas expuestas a los machos, es la intensidad del comportamiento sexual de los machos (Flores et al., 2000; Perkins y Fitzgerald, 1994; Rosa et al., 2000). En efecto, una mayor proporción de hembras incrementan las concentraciones plasmáticas de LH, presentan estro y ovulan cuando estas son expuestas a machos que despliegan un intenso comportamiento sexual, en comparación con aquellas que están en contacto con machos que despliegan un débil comportamiento sexual (Delgadillo et al., 2001, 2002; Flores et al., 2000; Rosa et al., 2000; Perkins y Fitzgerald, 1994). Por ejemplo, una baja proporción de cabras ovula cuando son expuestas a machos cabríos en reposo sexual. En cambio, la gran mayoría (>90%) de la hembras caprinas ovula cuando son expuestas a machos caprinos inducidos a un intenso comportamiento sexual al exponerlos a 2.5 meses de días largos artificiales a partir del 1 de noviembre, seguidos del fotoperiodo natural o implantes subcutáneos de melatonina (Flores et al., 2000; Loya-Carrera et al., 2014; Bedos et al., 2012). En conjunto, estos resultados sugieren que en caprinos, los machos fotoestimulados que despliegan intenso comportamiento sexual son muy eficaces para estimular la actividad ovulatoria de las cabras durante el anestro estacional.

4.8 Fertilidad de las cabras expuestas a los machos cabríos fotoestimulados

En la mayoría de los estudios realizados en el norte de México, la tasa de gestación promedio registrada en cabras expuestas por 15 días a machos fotoestimulados es del 75% (rango del 58% al 81%; Bedos et al, 2010; Fitz-Rodríguez et al., 2009). Parte de las hembras que no quedan gestantes en la segunda ovulación inducida por los machos pueden mostrar una fase lútea de duración normal, y luego entrar en anovulación, incluso cuando los machos permanecen con las hembras durante 35 días consecutivos (Flores et al., 2000). Otras cabras ovulan y muestran un comportamiento estral entre 24 y 28 días después de la segunda ovulación inducida por los machos (Delgadillo-Sánchez et al., 2003; Ott et al., 1980).

4.9 Posibilidades de incrementar la fertilidad de las hembras caprinas expuestas al efecto macho

La variabilidad de la tasa de gestación registrada con la presencia de los machos por 15 días, sugiere que existe un margen para mejorar la tasa de gestación. Un factor que podría mejorar la tasa de gestación, es la introducción de un macho nuevo al días 23 después del primer contacto entre sexos, para estimular las ovulaciones de las hembras que entran en anestro, o fecundar las hembras que presentan celo entre 24 y 28 días de iniciado el efecto macho. Por ello, considerando el efecto positivo de la novedad de los machos sobre la ovulación en las ovejas (Hawken et al., 2009; Jorre de St Jorre et al., 2012), y la capacidad de los machos cabríos fotoestimulados para inducir la actividad sexual de las cabras anéstricas, el objetivo del presente estudio es determinar si la introducción de machos cabríos “nuevos” el día 23 después del primer contacto con otros machos, permite incrementar la tasa de gestación en un grupo de cabras cuando se realiza un efecto macho durante 35 días.

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V. Artículo

Male effect in goats from subtropics

Introduction of novel bucks at day 23 of a male effect does not improve further pregnancy rate in goats

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Abstract

The ovulation can by induced by the introduction of a male among a group of anovulatory goats, by the "male effect". The objective of this study was to determine whether changing male goats for novel ones on day 23 after the initial introduction of males, would improve the pregnancy rate in goats exposed for 35 days to bucks. We used sexually active bucks, which were put in contact (n=2 each) with three groups of goats (n=30 each): i) males remained with females for 15 days (control group); ii) males remained with females for 35 days (same buck group); iii) males were removed at day 23 and replaced by other males which were not familiar to the females and which remained with females until day 35 (new buck group). Percentage of goats ovulating did not differ among groups at day 19 (≥87%; P=0.12). Pregnancy rates at day 35 did not differ between groups (≥70%; P=0.90). At day 55, pregnancy rates were similar between same and new buck groups (96%; P=1.0), but were higher than that of the control group (73%; P<0.05). Therefore, changing bucks for new ones at day 23 after the onset of the male effect did not increase the pregnancy rates compared with females that remained with the same bucks for 35 days. However, maintaining bucks with females for 35 days significantly increased pregnancy rates of more than 20 points, compared to those of goats that remained in contact with males for 15 days only.

Keywords: Caprine, Novelty of Males, Photoperiod, Ovulation, Sexual behavior.

Introduction

In sheep and goat breeds that show reproductive seasonality, the introduction of a male in a group of seasonally anestrous females can induce their reproductive activity within a few days after joining. In sheep and goats, this phenomenon of sexual stimulation is known as the male effect (Shelton, 1960; Delgadillo et al. 2009; Ungerfeld et al. 2004). The male effect is traditionally carried out in spring or summer, one month before females start their seasonal reproductive activity, thus permitting an advance of the natural onset of seasonal reproduction (Fernandez et al. 2011; Zarazaga et al. 2011; Abecia et al. 2015).

In goats, this early onset of reproduction results from the fact that the sudden introduction of a male induces and synchronizes the preovulatory LH surge, ovulations and the estrous behavior within the following 5 days (Bedos et al. 2014; Martínez-Alfaro et al. 2014; Zarazaga et al. 2014). Furthermore, in goats, this male effect can be obtained at any time during the anestrous season if males are rendered sexually active by an adequate photoperiodic treatment (Flores et al. 2000; Ponce et al. 2014; Chasles et al. 2016). In this species, the first male-induced ovulation occurs 2 to 5 days after the introduction of males and is associated with estrous behavior in a variable number of goats (Ott et al. 1980; Chemineau 1987). However, regardless of the presence or not of estrus, this ovulation is rarely fertile because after this first ovulation, most females display an ovulatory cycle of short duration and ovulate again 6 to 9 days later. This second ovulation is generally associated with estrous behavior and is fertile in most females (Ott et al. 1980; Walkden-Brown et al. 1999; Chemineau et al. 2006). Therefore, most females can become pregnant at the time of the second male-induced ovulation, within twelve days after introducing the bucks (Flores et al. 2000; Delgadillo-Sánchez et al. 2003; Chemineau et al. 2006).

In most of the studies performed in our group, the mean pregnancy rate registered in goats exposed to photostimulated males for 15 days is around 75%, ranging from 58% to 81% (Fitz-Rodríguez et al. 2009; Bedos et al. 2012; Loya-Carrera et al. 2014). Part of the females that do not become pregnant on the second ovulation display a luteal phase of normal duration, and then enter in anovulation. The other part of goats ovulate and display estrous behavior between 24 and 28 days after the second ovulation induced by males (Flores et al. 2000; Delgadillo-Sánchez et al. 2003). The variability of the pregnancy rate achieved at 15 days after the onset of the male effect still leaves margin for improvement. One simple possibility to achieve a higher rate of pregnancy would be to leave the buck in contact with females until the second fertile ovulation occurring around days 26-33 following male introduction.

Another factor to further improve the reproductive success of the ovulatory response of anestrous females to the male effect is the novelty of the male stimulus, which could be achieved by introducing a male unfamiliar (novel) to females in the middle of the male effect. Indeed, in sheep 75% of ewes displayed a LH surge and ovulated when exposed to novel rams, whereas only 25% of ewes did so when exposed to familiar rams, i.e. males already known by females (Jorre de St Jorre et al. 2012). Considering this positive effect of novelty of males on ovulation and estrous behavior, we hypothesized that: i) the prolongation of contact between males and females for 35 days could improve pregnancy rates compared with goats remaining with males for 15 days; ii) the improvement of pregnancy rate could be higher changing bucks for novel ones at day 23 of male stimulation.

Material and methods

General conditions of study

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 (SAGARPA, NOM-062-ZOO-1999).

The current study was conducted using local goats (*Capra hircus*) of the Laguna region in the state of Coahuila, Mexico (latitude 26º 23 'N and longitude 104 ° 47' W). Animals were 3 to 4 years old at the beginning of the study. In females from this region, seasonal anestrus occurs from March to August, whereas in males, the sexual rest lasts from January to May (Delgadillo et al. 1999; Duarte et al. 2008). All animals were fed with alfalfa hay ad libitum and 200 g of commercial concentrate (14% crude protein, 2.3 Mcal/kg). Animals had free access to water and minerals during the study.

Photo-induction of sexual activity of male goats

Eight bucks were kept together in an open pen (6 x 6 m) and subjected to a treatment of artificial long days (16 h light per day and 8 hours of darkness) from November 1st to January 15th. On January 16th, the photoperiodic treatment was suspended and males were exposed to the natural variations of the photoperiod until the end of the study, on May 7th. This photoperiodic treatment stimulates secretion of testosterone and improves the sexual behavior of males from late February to early May, months corresponding to the natural period of sexual rest (Delgadillo et al. 2002; Rivas-Muñoz et al. 2007; Ponce et al. 2014).

Preparation of Females

All females of the present study had given birth between August and September and were milked manually once daily during the study. On March 1st, 10th and 19th, the ovulatory activity of 90 multiparous goats was assessed by transrectal ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe (Ginther and Kot 1994; Delgadillo et al. 2011). Taking into account that in goats the follicular phase lasts 2-4 days the observation of the ovaries by transrectal ultrasonography is an efficient method to identify females in anovulatory state (Fatet et al. 2011). Therefore, females with the absence of corpora lutea in the three observations were considered to be in seasonal anovulatory state (Delgadillo et al. 2011). The 90 females evaluated by ultrasonography were diagnosed as anovulatory. On March 26th, these females were divided into 3 homogenous groups (n=30 each) balanced for body condition score (BCS; 1: very lean, 4: fat; Table 1; Walkden-Brown et al. 1997). Each group was kept in different shaded open pens (8 x 8 m).

Male effect

We used three groups of seasonally anovulatory goats to test the effect of new bucks on the efficiency of the male effect, we used three groups of seasonally anovulatory goats. On March 29th, each group of females was exposed to the photostimulated male goats (n=2 each). Goats from the first group were exposed to the photostimulated males for 15 consecutive days (control group); goats from the second group were exposed to the photostimulated males for 35 consecutive days (same buck group); goats from the third group goats were exposed to males for 35 days, using two bucks that remained with the females for the first 23 days, and that were then exchanged for two novel bucks unknown to the females, and that stayed with them until day 35 (new buck group).

To explain possible differences in the ovulatory response of females, the sexual behavior of all bucks was assessed individually on day 0, i.e. at their introduction into the groups of females, on day 23 in the same and new male groups, just after the new bucks have been introduced in the new buck group. Trained persons directly observed and recorded during 30 min the frequency of nudging, a variable sufficiently reliable to assess the sexual behavior of bucks (Delgadillo et al. 2015).

Measurements

Ovulations were assessed by the presence of corpora lutea observed in each female by transrectal ultrasonography 19 days after introduction of the bucks in each group (Delgadillo et al. 2011). In addition, in the familiar and novel groups, the ovulatory activity was assessed by measuring the evolution of the plasma concentrations of progesterone. Thus, 5-mL blood samples were obtained every two days from day 23 to 33 after exposure to males to determine the ovulatory response to the introduction of the novel bucks. Blood samples were obtained from the jugular venipuncture in tubes containing heparin. Plasma was obtained after centrifugation at 3500 x g for

30 min and stored at -20°C. Concentrations of plasma progesterone were measured by immunoenzymatic assay as previously described by Canépa et al. (2008). Sensitivity of the assay was 0.25 ng/mL. The intra-assay coefficient of variation was 10.7%. Females with progesterone concentrations ≥1.0 ng/mL were considered to have ovulated (Delgadillo et al. 1998). Pregnancy rates were determined by the presence of embryos by transrectal ultrasonography 35 and 55 days after the initial introduction of bucks in each group (González de Bulnes et al. 1998).

Statistical analyses

The Chi-square test was used for multiple-group comparisons and the Fisher's exact probability test was used for two by two-group comparisons to determine statistical differences in the proportions of goats that ovulated and pregnancy rates. Analyses were performed using the statistical package SYSTAT 13.

Results

Females

The proportions of females that ovulated at day 19 after introduction of males did not differ significantly between the three groups (P=0.11; Table 1). Pregnancy rates at day 35 (reflecting eventual copulation between days 0 and 15) did not differ between the three groups (70 to 73%; P=0.90; Table 1). At day 55 pregnancy rates were similar between same buck and new buck groups (96% in both groups; P=1.0), but higher than that registered in control group at day 35 (96% vs. 73%, respectively; P=0.03; Table 1). Interestingly, 7 of 8 females from the same buck group that were not pregnant at day 35, ovulated again between days 23 and 35 and became pregnant at day 55 while one of them entered into anovulation (Figure 1). Similarly, 8 of 9 females from the new buck group that were not pregnant at day 35, ovulated between days 23 and 35 and became pregnant at day 55, and one of them entered in anovulation (Figure 1). Therefore, in the same buck and new buck groups, pregnancy rates increased significantly from day 35 to 55 (P=0.006; P= 0.01, respectively).

Table 1. Percentage of goats ovulating and pregnancy rates of seasonally anovulatory goats exposed to photostimulated, sexually active bucks. In the control group, the same bucks remained in contact with females for 15 days. In group 2, the same bucks remained in contact with females for 35 days. In group 3, bucks remained in contact with females for the first 23 days after their introduction, and then were exchanged with 2 new bucks which remained with females up to day 35. The initial introduction of males into the groups of females was on March 29th, during the seasonal anestrous. All males goats were rendered sexually active by exposure to artificial long days (16 h of day per day) from November 1st to January 15th, followed by natural photoperiod.

Groups Duration of contact	N	Ovulations day 19 (%)	Pregnancy rates day 35 (%)	Pregnancy rates day 55 (%)
Control -15 days	30	26/30 (87)	22/30 (73)	22/30 (73)[a]
Same bucks -35 days	30	30/30 (100)	22/30 (73)	29/30 (96)[b]
New bucks -35 days	30	28/30 (93)	21/30 (70)	29/30 (96)[b]

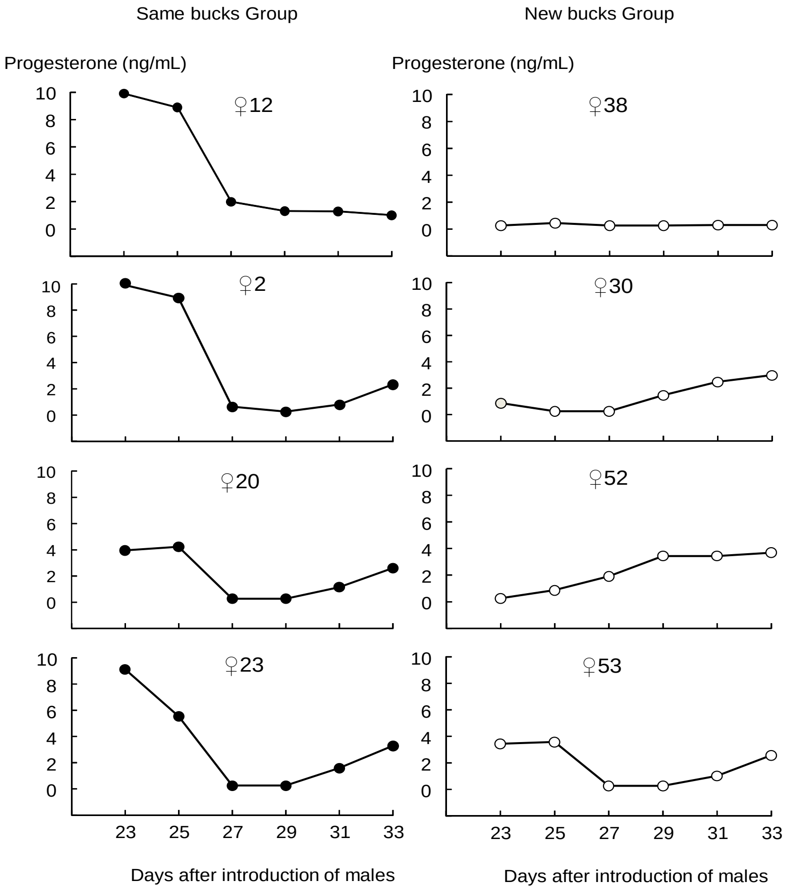

Figure 1

Figure 1. Representative profiles of plasma progesterone concentrations of goats exposed to photostimulated males goats. In one group, females remained with males from 35 consecutive days, the same bucks group (●). In the other group, males were removed at day 23 after the onset of contact between both sexes, and replaced by two other males, which remained with females until day 35, the new bucks group (○). All males were rendered sexually active by exposure to long days from November 1st to January 15th followed by natural photoperiodic conditions.

Males

At day 0, the total number of nudgings displayed by the two males from the control (72), new buck (61) and same buck (153) groups was high. Similarly, at day 23, the total number of nudging displayed by males from the new buck (85) and same buck (56) groups was high.

Discussion

The results of this study show that maintaining a buck among females until day 35 following its introduction during a male effect improves significantly reproductive rates. However, our results do not support our initial hypothesis, that changing bucks for novel ones at day 23 would improve the pregnancy rate. It appears therefore that maintaining the same males for 35 days was sufficient for females to keep cycling until their third estrus and ovulation and then to fertilize them at this third ovulation. Rather, the continuous presence of males, regardless of their exchange, allowed males to mate females displaying estrus and ovulations around 24 and 28 days after introducing the males, increasing therefore the pregnancy rates at day 55 compared to those of females maintained with bucks for only 15 days.

The difference of pregnancy rates observed at day 55 between females that remained with bucks for 15 days and females that remained with bucks for 35 days cannot be explained by an initial difference of response to the males between groups, since the percentages of females that ovulated at day 19 after introducing the males were ≥87%, and did not differ between groups. The present results also confirm previous results reported in goats exposed to photostimulated males and showing that sexually active bucks can induce an efficient male effect even in the middle of seasonal anestrus (Bedos et al. 2012; Loya-Carrera et al. 2014; Ponce et al. 2014). Also, the lower pregnancy rate following 15 days of contact does not appear related to differences of fertility at the second ovulation, since the pregnancy rates at day 33 were similar in the three groups.

Rather, one likely explanation for this difference between 15 and 35 days of contact is that with a longer contact, males can fertilize females on their third ovulation. These females had not been fertilized during their previous cycle, either because they had not been detected in estrus by the bucks, or because females were mated but no fertilized. The opportunity of a third cycle for 30% or so of females that had not been fertilized permitted the males to mate and compensate for the females that were not mated.

Finally, it is clear that the novelty of males had not any impact on the female reproductive performances. This lack of effect of new bucks suggests that familiarity had no impact on female response. Interestingly we have obtained results, concomitantly to the present experiment, showing that the introduction of novel buck to female goats does not improve the female response to the male effect (Muñoz et al. 2016). However, it should be noticed that this result was obtained with a male effect lasting 15 days and that a putative differential response according to familiarity was not tested after this period. In addition, the lack of difference could also be due to the fact that after day 23, the percentage of females being pregnant is already quite high (>73%) and therefore a potential significant effect of the introduction of novel males is quite hardly observable. This issue would be only testable by using a much larger number of animals.

Overall, we can conclude that in our conditions of study, novelty of males in the middle of the male effect is not critical to ensure a very high reproductive efficiency, whereas increasing duration of contact is important, at least when a ratio of 1 male for 15 females is used. Whether using a higher ratio of males would permit to reduce the duration of contact while keeping a pregnancy rate of about 90% remains to be investigated.

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VI. Conclusión

Los resultados del presente estudio demuestran que la introducción de machos cabríos nuevos al día 23 de iniciado el efecto macho no aumenta la tasa de gestación de las cabras. Sin embargo el mantener ambos sexos en contacto directo por un periodo de 35 días, aumenta significativamente el porcentaje de la tasa gestación de la cabras, comparado con aquellas cabras que se exponen a machos fotoestimulados únicamente durante 15 días.

VII. Literatura Citada

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