Two sexually active bucks housed with four successive groups of anovulatory does induced high pregnancy rates during seven-day mating period

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Short Note [Nota corta]

TWO SEXUALLY ACTIVE BUCKS HOUSED WITH FOUR SUCCESSIVE GROUPS OF ANOVULATORY DOES INDUCED HIGH PREGNANCY RATES DURING SEVEN-DAY MATING PERIODS †

[DOS MACHOS CABRÍOS SEXUALMENTE ACTIVOS ALOJADOS CON CUATRO GRUPOS SUCESIVOS DE HEMBRAS ANOVULATORIAS INDUJERON ALTAS TASAS DE PREÑEZ DURANTE PERIODOS DE EMPADRE DE SIETE DÍAS]

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SUMMARY

Background. In seasonally anovulatory goats, sexually active bucks led a high pregnancy rate during a mating period of 36 days. Progesterone priming of does can reduce the length of the mating period while maintaining high fertility by allowing oestrous behaviour at the first male-induced ovulation. **Objective.** To determine whether high fertilization rates could be achieved by using two sexually active bucks, alternated daily for a period of seven days in four successive groups of does. **Methodology.** Two control bucks were used only with one group of does (n = 19), whereas two experimental bucks were successively housed with four groups of does (n = 18 or 19 each). One of the control and experimental bucks was introduced to their respective groups in the morning and removed 24 h later to rest for 24 h. Immediately after the removal of the first buck, the second buck was placed with the group of does. Each doe was treated with 25 mg of progesterone im 48 h prior to the first introduction of bucks to reduce short ovulatory cycles. **Results.** In each group, the proportion of does that ovulated was higher than 93%. These proportions did not differ among groups exposed to the control or experimental bucks (P > 0.05). In each group, the proportion of pregnant does was higher than 78%; proportions did not differ among groups (P > 0.05). **Implications.** At farms with few bucks, efficient management of sexually active bucks can yield a high percentage of pregnant females. **Conclusion.** These results indicate that two sexually active bucks housed with four successive groups of progesterone-treated anovulatory does during a mating period of seven days were able to achieve high pregnancy rates.

Key words: caprine; anovulatory does; hormonal treatment; light treatment; male effect; male-female ratio; sexual behavior; subtropics.

RESUMEN

Antecedentes. En cabras en anovulación estacional, los machos cabríos sexualmente activos permiten obtener altas tasas de preñez en un período de apareamiento de 36 días. El tratamiento de las hembras con progesterona antes de ponerlas en contacto con los machos reduce la duración del período de apareamiento sin modificar la fertilidad al permitir el comportamiento estral en la primera ovulación inducida por el macho. **Objetivo**. Determinar si se pueden lograr altas tasas de preñezs utilizando dos machos cabríos sexualmente activos, alternados diariamente durante un período de siete días en cuatro grupos sucesivos de hembras. **Metodología**. Se usaron dos machos testigo que se introdujeron solamente con un grupo de hembras (n = 19), mientras que dos machos experimentales se introdujeron sucesivamente con cuatro grupos de hembras (n = 18 o 19 cada uno). Uno macho testigo y otro experimental se introdujeron a sus respectivos

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grupos en la mañana y se retiraron 24 h después para descansar por 24 h. Inmediatamente después de sacar el primer macho, el segundo macho se introdujo con el grupo de hembras. Cada hembra fue tratada con 25 mg de progesterona im 48 h antes de la primera introducción de los machos para reducir los ciclos ovulatorios de corta duración. **Resultados**. En cada grupo, la proporción de hembras que ovuló fue superior al 93% y no hubo diferencias entre los grupos expuestos a los machos testigo o experimentales (P > 0.05). En cada grupo, la proporción de hembras preñadas fue superior al 78% y no hubo diferencias entre los grupos expuestos a los machos testigo o experimentales (P > 0.05). **Implicaciones**. En hatos con pocos machos, el manejo eficiente de los machos sexualmente activos puede permitir obtener altos porcentajes de cabras preñadas. **Conclusión**. Estos resultados indican que dos machos sexualmente activos puestos en contacto con cuatro grupos sucesivos de hembras anovulatorias tratadas con progesterona durante un período de apareamiento de siete días pudieron lograr altas tasas de preñez.
Palabras clave: caprinos; cabras anovulatorias; tratamiento hormonal; tratamiento luminoso; efecto macho; relación macho-hembras; comportamiento sexual; subtrópicos.

INTRODUCTION

The sexual response of does exposed to bucks may be influenced by the intensity of the males' sexual behaviour. Bucks made sexually active by exposure to photoperiodic treatments are more efficient than untreated control bucks at stimulating sexual activity in anovulatory does (Chasles *et al*., 2016; Zarazaga *et al*., 2019). Another factor that may influence the response of ewes and does to males is the male:female ratio. In sheep, a reduction in the male:female ratio from 1:25 to 1:100 results in a significant decrease in the proportion of ewes ovulating or resulting lambs (Lightfoot and Smith, 1968; Signoret *et al*., 1982). In contrast, in goats, reducing the male:female ratio from 4:39 to 1:39 did not reduce the proportion of ovulating does when sexually active males were used (Carrillo *et al*., 2007). However, this latter study did not report the proportions of goats that exhibiting oestrus or becoming pregnant. In most studies using sexually active bucks, the male:female ratio is ~1:10, resulting in ~75% of the does becoming pregnant during a mating period of 15 to 48 days (Loya-Carrera *et al*., 2014; Chasles *et al*., 2016; Zarazaga *et al*., 2018). This male:female ratio underutilizes males, considering that the optimal sex ratio is 1:20 with a mating period of 36 days, resulting in a fertility of 60% (Zarazaga *et al*., 2018).

Considering that i) sexually active goat bucks are very efficient in stimulating reproductive activity in seasonally anovulatory does at a male:female ratio of 1:10, ii) progesterone treatment prior to male introduction reduces short ovulatory cycles and induces first ovulations associated with oestrous behaviour in the first seven days after male introduction, and iii) a male:female ratio of 1:20 is optimal with a mating period of 36 days, we hypothesized that daily alternation of the same two sexually active bucks could be able to induce high pregnancy rates in four consecutive groups of progesterone-treated goats with a mating period of seven days at a male:female ratio of 1:18 or 19. To test this possibility, two bucks were used successively with four groups of does, and the results were compared with those of other two bucks used only with one group of does.

MATERIALS AND METHODS

Study conditions

The study was carried out in the Laguna region of the state of Coahuila, Mexico (latitude 26°23' N; longitude, 104°47' W). In this region, the day length varies from 13 h 41 min (at the summer solstice) to 10 h 19 min (at the winter solstice). The goats used in this study were of Spanish breeds Granadina, Murciana and Malagueña. These animals have been crossed with Alpine, Saanen, Toggenbourg and Anglo-Nubian breeds in the last 65 years to improve milk and meat production (Delgadillo, 2011). In females of this population, seasonal anovulatory lasts from March to August; in bucks, seasonal sexual rest lasts from January to June. These seasonal variations in sexual activity in females and males mainly depend on the annual variation in day length (Delgadillo *et al*., 2004; Duarte *et al*., 2010). The goats used in the present study (n = 92) came from the same flock and were four years old at the beginning of the study. The females were multiparous, had given birth between October and December prior to the study and were milked manually every morning during the study. The male goats (n = 8) were five years old and were separated from does from October until the onset of the study. The bucks and does were fed two kg of alfalfa hay/animal/day (9.6 MJ/kg, 18% crude protein/kg of dry matter; National Research Council, 2007) and had free access to water and mineral blocks.

Stimulation of buck sexual activity by exposure to artificially long days

The bucks were rendered sexually active during their seasonal sexual rest by a photoperiodic treatment. Briefly, all bucks were exposed to artificially lengthened days (16 h of light/8 h of darkness) from 15 November to 15 January in an open pen (6 × 6 m). Starting on 16 January, the bucks were exposed to the natural photoperiod until the end of the study. This photoperiodic

treatment enhances plasma testosterone concentrations, sexual behaviour and sperm production during approximately two months (in the present study: March and April, normally the period of seasonal sexual rest; Delgadillo *et al*., 2002). On 20 and 21 March, semen was collected using an artificial vagina when the males were presented to an intact oestrous-induced doe (Delgadillo *et al*., 1999). Then, the total number of spermatozoa per ejaculate (volume × sperm concentration of the ejaculate) was assessed immediately after semen collection using a photometer (Goat SpermaCue; Minitübe, Tiefenbach, Germany). All males produced more than 3.2×10^9 spermatozoa per ejaculate. On 22 March, the sexual behaviour of the bucks was determined individually in a 15-min test. Every buck was exposed to an anovulatory doe, and the number of nudges was recorded as a reliable variable of the sexual activity of the bucks. Out of eight bucks exposed to artificially long days, four were selected considering their similar sperm production and sexual behaviour and further divided into the control and experimental groups (n = 2 each).

Conditions of the female goats

The female goats were anovulatory at the beginning of the study. Anovulation was determined in each female by transrectal ultrasonography performed on 3, 13 and 23 March using an Aloka SSD-500 device (Aloka Co., Ltd, Tokyo, Japan) connected to a transrectal 7.5-mHz linear probe (Simões et al., 2007). Females without corpora lutea at the three ultrasounds were considered to be in seasonal anovulation. On 24 March, anovulatory females were assigned to five groups according to their body condition, which was determined by using a scale from 1 (very lean) to 4 (obese) based on lumbar palpation (Table 2; Walkden-Brown et *al*., 1997), and allocated to shaded open pens (one pen per group).

The male effect

The two control bucks were housed with one group of females (n = 19) for seven days beginning on 16 April 2018. The two experimental bucks were successively housed with four groups of females (seven days per group; n = 18 or 19 each) beginning on 26 March of the same year. When experimental males were housed with the fourth female group, the control males were housed with their respective group. Therefore, we used a male:female ratio of 1:18 or 19 throughout the study. This design allowed us to determine whether the mating activity of the experimental bucks with the three previous groups of females decreased their fertility. Thus, one control or experimental buck was added to its respective group in the morning, remained with the does for 24 h and was removed from the group the next morning. Immediately after removal, the other control or experimental buck was introduced to the same group of females. This procedure was performed throughout the study, such that every buck was housed with the females of each group for three days, alternating with resting days. During resting days, the bucks were placed in individual pens adjacent to the doe pen until the next day. The distance between does housed with males and the other groups of does waiting to be housed with males was more than 100 m to prevent possible stimulation of sexual activity. All females were treated with 25 mg of progesterone IM (Facilgest 25 mg/mL; Syva laboratories) on the right side of the neck 48 h prior to buck introduction to reduce or prevent the occurrence of short ovulatory cycles (Lasssoued *et al*., 1995; Gonzalez-Bulnes *et al*., 2006). Considering our previous results with sexually active bucks (Bedos *et al*., 2010; Loya-Carrera *et al*., 2014), we estimated that the number of males and females used in each group would allow the observation of significant differences in the sexual behaviour of males and the ovulatory response and pregnancy rates of females.

Measurements

The sexual behaviour of the bucks was observed for two days after their introduction to the female groups. Each day, the sexual behaviour of the bucks was observed by a trained experimenter from 08:00 to 08:30, and the number of nudges was recorded. Ovulations were determined by the presence of corpora lutea observed by transrectal ultrasonography in each female 18 days after the first exposure to the bucks. Pregnancy rates (pregnant females/females exposed to bucks) were determined by transrectal ultrasonography of the females 45 days after the first exposure to the bucks.

Statistical analyses

The number of nudges by the bucks was analysed using the chi-squared test of independence. For experimental males, the variation in the number of nudges over time (G1 to G4) was analysed using the same statistical procedure described above. The proportions of females that ovulated and became pregnant were analysed using the chi-squared test for multiple-group comparisons. Body condition of females was analysed using the Kruskal–Wallis test. All data were analysed using the System Statistics (2009).

RESULTS

Sexual behaviour of the bucks

Experimental bucks displayed more nudges when exposed to G3 and G4 does than control bucks (P

< 0.05). However, there was no difference in nudges between the experimental bucks housed with G1 and G2 does and the control bucks (P > 0.05). Furthermore, the number of nudges displayed by the experimental bucks increased over time; this number was higher when housed with G2, G3, and G4 does than when housed with G1 does (P < 0.01; Table 1).

Table 1. Total number of nudges displayed by control and experimental goat bucks in the presence of does. All bucks were rendered sexually active by exposure to artificially lengthened days (16 h of light/8 h of darkness per day) from 15 November to 15 January. Afterwards, the bucks were exposed to the natural photoperiod until the end of study.

Groups of males	*n*‡	Total number of nudges
Control	2	237[a]
Experimental		
G1	2	[A] 185 [a]
G2	2	[B] 267 [a]
G3	2	[B] 316 [b]
G4	2	[B] 364 [b]

[a,b] Lowercase letters within a column indicate significant differences in the number of nudges between the control and the four experimental groups ($P < 0.05$).
[A,B] Uppercase letters within a column indicate significant differences in the number of nudges between the four experimental groups ($P < 0.01$).
‡ The two experimental bucks were successively housed with four groups of females (seven days per group).

Ovulatory response to male introductions and pregnancy rates

The proportions of does that ovulated did not differ between groups housed with experimental or control bucks ($P > 0.05$), and more than 93% of the does ovulated in each group. Pregnancy rates did not differ between groups housed with experimental or control bucks ($P > 0.05$), and more than 78% of the does in each group became pregnant (Table 2).

DISCUSSION

The results of the present study showed that daily alternation between the same two sexually active bucks stimulated reproductive activity in four groups of seasonally anovulatory goats as effectively as daily alternation of two sexually active bucks in contact with only one group of females with a seven-day breeding season. Thus, using a daily buck:doe ratio of 1:18 or 19 (instead of 1:10) maintained high ovulatory responses and pregnancy rates. In fact, the proportions of females ovulating in each group was greater than 93% and did not differ between females that mated with experimental or control bucks. In addition, and more importantly, the pregnancy rates were higher than 78% and did not differ between females that mated with experimental or control bucks. Taken together, these results confirm our hypothesis that daily alternation between the same two sexually active bucks can lead to successful fertilization of four groups of progesterone-treated anovulatory females with a male:female ratio of 1:18 or 19 and a seven-day mating period in each group.

Ovulatory responses and pregnancy rates in does housed with the experimental bucks can be explained by several nonexclusive hypotheses. Firstly, females in the present study were treated with 25 mg of progesterone to trigger oestrous behaviour at the first male-induced ovulation and to induce a normal luteal phase, thus allowing fertile copulations in the first seven days after male introduction (Lassoued *et al*., 1995; Gonzalez-Bulnes *et al*., 2006). Thus, the proportions of pregnant females that mated with the experimental bucks did not differ from those that mated with the control bucks, indicating that the mating activity of the experimental bucks

Table 2. Ovulations and pregnancy rates of anovulatory does housed with control or experimental bucks. The two control bucks were housed with one group of does for seven days. The two experimental bucks were successively housed with four groups of females for seven days per group. All bucks were rendered sexually active by exposure to artificially lengthened days (16 h of light/8 h of darkness per day) from 15 November to 15 January. Afterwards, the bucks were exposed to the natural photoperiod until the end of study.

Goats housed with bucks	*n*	*BC (Mean ± SEM)	Goats with ovulations (%)	Pregnancy rates (%)
Control	19	1.6 ± 0.1 [a]	18/19 (95) [a]	15/19 (79) [a]
Experimental				
G1	18	1.5 ± 0.1 [a]	17/18 (94) [a]	15/18 (83) [a]
G2	18	1.4 ± 0.1 [a]	18/18 (100) [a]	16/18 (89) [a]
G3	18	1.5 ± 0.1 [a]	17/18 (94) [a]	15/18(83) [a]
G4	19	1.5 ± 0.1 [a]	19/19 (100) [a]	16/19 (84) [a]

[a]Similar superscripts within each column indicate no significant differences among groups ($P > 0.05$).
*Body Condition: scale 1 (very lean) to 4 (obese).

with the three previous groups of females did not reduce their fertility. Secondly, in the current study, the bucks were alternated daily, which allowed them to recover both sperm production and sexual behaviour during the 24-h rest period, enabling them to fertilize more females. Another possibility is that in our study, each reintroduction of bucks to each group of females increased their mating activity, as previously reported (Bedos *et al*., 2012). Finally, it is interesting to note that the proportions of females that ovulated and became pregnant when exposed to the experimental or control bucks were similar to those reported with a male:female ratio of ~1:10 and a mating period of 15 days using sexually active bucks (Bedos *et al*., 2012; Loya-Carrera *et al*., 2014). We can therefore conclude that daily alternation of bucks allowed them to fertilize more progesterone-treated does.

The experimental bucks displayed more nudges when housed with G3 and G4 does than the control bucks. This result suggests that the sexual behaviour of the control and experimental bucks was similar at the beginning of the study. Interestingly, among the experimental bucks, the number of nudges increased over time; more nudges were observed when the bucks were housed with G2, G3, and G4 females than when the bucks were housed with G1 females. It is likely that the interaction of experimental bucks with a greater number of new females increased nudging behaviour, as previously reported (Bedos *et al*., 2012). Another explanation for this finding is that the experimental bucks rested for 24 h, and in each reintroduction into the groups of females, the females in oestrous may have stimulated the sexual behaviour of the bucks via the "female effect" (Walkden-Brown *et al*., 1994). A complementary reason for this increased sexual behaviour in males could be based on a kind of "Coolidge effect", where the presentation of new sexual partners stimulates sexual behaviour (Brown, 1974). Taken together, our results showed that nudging increased over time, allowing bucks to stimulate reproductive activity in four groups of seasonally anovulatory goats.

Replication of such experiments is difficult because of their cost and it can be argued that they cannot be extrapolated to the general goat population on farms. This is true for a direct inference. However, the demonstration here that only 2 bucks are able to induce ovulations in 71/73 goats and fertilize 62 of them in a short period of time, is clearly very positive and suggests that this experiment should be repeated on farms in the future to confirm that it can be used by goat farmers.

CONCLUSIONS

The results of the present study show that daily alternation between two sexually active males maintained high fertility rates in anovulatory goats when males were housed with four groups of does with a daily male:female ratio of 1:18 or 19 and a mating period of seven days. From a practical standpoint, our results indicate that on farms with few bucks, efficient management of sexually active bucks is key to achieving a high percentage of pregnant females.

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Conflicts of interest. The authors have no conflict of interest to declare.

Compliance with ethical standards. The experimental procedures used in the current study followed the technical specifications of the Official Mexican Rule for the production, care, and use of laboratory animals (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2001).

Data availability. The data that support the findings of this study are available from the corresponding author (joaldesa@yahoo.com) upon reasonable request.

Author contribution statement (CRediT). L.A. Espinoza-Flores, - Conceptualization, Investigation and Data curation; **J.A. Andrade-Esparza -** Conceptualization, Investigation and Data curation; **D. López-Magaña-** Conceptualization, Investigation and Data curation; **H. Hernández-** Conceptualization and Formal analysis; **R. J. Vielma -** Conceptualization and Methodology; **L. Zarazaga -** Conceptualization and Methodology; **M. Keller -** Conceptualization and Methodology; **P. Chemineau-** Conceptualization and Methodology; **J.A. Delgadillo-**Conceptualization, Supervision, Resources and Project administration; all authors participated in the Writing - original draft and Writing – review & editing.

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