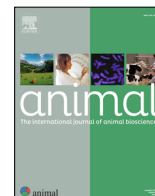




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Physical separation and reduction of contact duration with sexually hyperactive bucks decrease testosterone concentrations and sexual behaviour in bucks in sexual rest

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ABSTRACT

Sexually hyperactive bucks are more efficient than sexually hypoactive bucks in stimulating testosterone secretion and sexual behaviour in other bucks in seasonal sexual rest by the phenomenon that we called the “buck-to-buck effect”. Here, we determined whether physical separation and reduction of the duration of contact with the sexually hyperactive bucks would modify those parameters in sexually hypoactive bucks exposed to the “buck-to-buck effect”. Bucks were subjected to natural day length throughout the study; this was the sexually hypoactive group. Other bucks were subjected to artificial long days (16 h of light per day) from 15 November to 15 January followed by exposure to natural day length to stimulate their sexual activity during the rest season; this was the sexually hyperactive group. In Experiment 1, we determined testosterone concentrations and sexual behaviour of six sexually hypoactive bucks separated 1.5 m from six sexually hyperactive bucks for 60 days by a metal open work fence, while a control group of six sexually hypoactive bucks was in permanent contact with six sexually hyperactive bucks. In Experiment 2, the duration of contact with sexually hyperactive males was reduced from 31 days (contact group, six bucks) to 10 days (withdraw group, seven bucks). In experiments 1 and 2, there was an effect of time ($P < 0.01$) and an interaction between time and groups ($P < 0.05$). In Experiment 1, testosterone plasma concentrations were greater in bucks in contact with sexually hyperactive bucks than in those separated from bucks at 20 and 30 days after the introduction of sexually hyperactive bucks ($P < 0.01$). The bucks from the contact group also displayed more nudging than bucks from the separated group from 0 to 30 days ($P < 0.001$). In Experiment 2, testosterone concentrations were greater in the contact group than in those from the withdraw group from 19 to 31 days after the introduction of sexually hyperactive bucks ($P < 0.05$). Bucks from the withdraw group displayed more nudging than the contact group 7 days after the introduction of the sexually hyperactive bucks ($P < 0.05$). Afterwards, bucks from the contact group displayed more nudging than the withdraw group 14, 21 and 28 days after the introduction of the sexually hyperactive bucks. We concluded that physical separation and reduction of the duration of contact with the sexually hyperactive bucks decrease testosterone concentrations and sexual behaviour of bucks in sexual rest exposed to the “buck-to-buck effect”.

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Implications

In seasonal he-goats, sociosexual interactions between bucks modify the timing of the sexual season. Recently, we showed that the bucks made sexually hyperactive by a photoperiodic treatment

are more efficient than untreated ones to stimulate LH, testosterone secretion and sexual behaviour in bucks in seasonal rest. A phenomenon called the “buck-to-buck effect”. Here, we showed that physical separation and reduction of the duration of contact with sexually hyperactive bucks reduce testosterone secretion and sexual behaviour of stimulated bucks. The permanent presence of sexually hyperactive bucks is, therefore, necessary to fully stimulate the endocrine and sexual activities of bucks in sexual rest.

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Introduction

The introduction of a buck or ram into a group of females in seasonal anestrus immediately stimulates the secretion of LH, leading to ovulation within 5 days after contact (Chemineau, 1983; Ungerfeld et al., 2004; Bedos et al., 2014) a phenomenon known as the “male effect” (Walkden-Brown et al., 1999; Delgadillo et al., 2009). The secretion of LH and ovulation of females exposed to the male effect can be influenced by the physical separation from males and the duration of contact with males. In goats and ewes, the ovulatory response is lower in females separated from males by a wire fence or a passage than in those in full physical contact with them (Chemineau, 1987; Shelton, 1980; Pearce and Oldham, 1988). Similarly, in goats and ewes, exposure to males increases secretion of LH within 15 min, but as soon as the male is withdrawn, secretion of LH decreases (Oldham and Pearce, 1983; Bedos et al., 2014). In the same way, the ovulatory activity in response to males varies with the duration of male contact, and the percentage of females that ovulated is lower when joined with males for 4 days than in those joined with them for 15 days (Signoret et al., 1982). The intensity of sexual behaviour displayed by males is another factor that strongly influences the secretion of LH and the ovulatory response to males. Indeed, bucks or rams rendered sexually hyperactive during the sexual rest by photoperiodic treatments are more efficient in spring to stimulate LH secretion and ovulations than untreated, sexually hypoactive males (Abecia et al., 2016; Chasles et al., 2016; Zarazaga et al., 2019).

As in females, the sexually hyperactive bucks or rams are more efficient than sexually hypoactive males to stimulate LH and testosterone secretion, and to improve sexual behaviour and sperm production in other males in seasonal sexual rest by the phenomenon that we called the “male-to-male effect” (Abecia et al., 2022; Delgadillo et al., 2022). This phenomenon was described when sexually hyperactive and sexually hypoactive bucks were housed in the same pen, allowing continuous physical contact and free interactions between them. As a whole, these new elements concerning the importance of socio-sexual relationships between sexes and within sexes which were able to completely neutralize the classical photoperiodic seasonal inhibition on reproductive activity (Delgadillo et al., 2015), have led to question the equilibrium and relative importance of photoperiod and sexual relationships in seasonal reproduction of small ruminants (Delgadillo et al., 2022).

Considering that in seasonally anestrus goats, physical separation from bucks and reduction of the duration of contact with them decreased LH and ovulatory responses to males, we hypothesized that the physical separation and reduced time of contact with the sexually hyperactive bucks would decrease testosterone plasma concentrations and sexual behaviour of sexually hypoactive bucks exposed to the “buck-to-buck effect”. To test this possibility, we performed two experiments. In Experiment 1, we determined testosterone concentrations and sexual behaviour of sexually hypoactive bucks separated 1.5 m from sexually hyperactive bucks, while a control group of sexually hypoactive bucks was in permanent contact with sexually hyperactive bucks. In Experiment 2, we determined the testosterone concentrations and sexual behaviour of sexually hypoactive bucks when the duration of contact with sexually hyperactive males was reduced from 31 to 10 days.

Material and methods

General study conditions

The study was performed in the Laguna region in the state of Coahuila in the north of Mexico (Latitude 26° 23' N and Longitude 104° 47' W). In this region, the duration of daylight varied from

13 h 41 min at the summer solstice to 10 h 19 min at the winter solstice. We used bucks from the local population of the Laguna region, whose origin and physical characteristics were already described (Duarte et al., 2008). In bucks of this population, sexual rest lasts from January to May (Delgadillo et al., 1999). The bucks were kept in open pens and were fed 2 kg of alfalfa hay (17% CP) and 100 g of commercial concentrate (9.6 MJ/kg and 14% CP per kg of DM) per day throughout the study, and had free access to water and mineral blocks. In both experiments, some measurements and testosterone assays, experimental conditions (experimental facilities), and statistical analyses are identical to those used in previous experiments (Delgadillo et al., 2022 and 2024). Consequently, the description of some methodologies is identical to that found in both publications.

Experiment 1. Effects on testosterone secretion and sexual behaviour of sexually hypoactive bucks separated 1.5 m from sexually hyperactive bucks

Sexual stimulation of bucks by a photoperiodic treatment

The bucks were 4 years old at the beginning of the study. On 1 November, bucks were allocated into two groups with similar body and testicular weights assessed by comparative palpations with an orchidometer (Oldham et al., 1978). Bucks from one group ($n = 12$) were subjected to natural day length throughout the study; this was the sexually hypoactive group (BW: 40 ± 4 kg; testicular weight: 80 ± 8 g; mean \pm SEM). Bucks from the other group ($n = 6$) were subjected to artificial long days (16 h of light per day) from 15 November to 15 January followed by exposure to natural day length to stimulate their sexual activity during the rest season (Delgadillo et al., 2021); this was the sexually hyperactive group (BW: 42 ± 3 kg; testicular weight: 85 ± 7 g).

Experimental design

On 1 April, sexually hypoactive bucks were allocated into two groups ($n = 6$ each) with similar body and testicular weights. On 6 April (Day 0), one group of bucks was joined with two sexually hyperactive bucks during 60 days; this was the contact group (BW: 40 ± 5 kg; testicular weight: 82 ± 8 g). The other group was separated 1.5 m from these bucks by a metal openwork fence that allowed visual, olfactory, and auditory contact between bucks; this was the separated group (BW: 43 ± 3 kg; testicular weight: 85 ± 6 g).

Measurements

Jugular blood samples were collected in tubes containing 30 μ L of heparin on day 0 just before introducing the bucks, and then on day 1 and every 10 days until 60 days after introducing the bucks to determine plasma testosterone concentrations. Plasma was obtained after centrifugation at 3 500 g for 30 min and stored at -20°C until assayed by a direct enzyme immunoassay according to Delgadillo et al. (2024). Sensitivity was 0.15 ng/mL, and the intra-assay CV was 8.2%. Sexual behaviour displayed by the bucks of the contact and separated groups was determined for 10 min after introducing the bucks, and then every 10 days throughout the study as recently described (Delgadillo et al., 2022). For this purpose, the number of nudging events was determined. Nudging consists in lateral approach of the male flexing its foreleg against the males with short, choppy kicking motions with or without extension and retraction of the tongue and low-pitched vocalizations. This definition was modified from that used by Bedos et al. (2016) in females.

Experiment 2. Effects on testosterone secretion and sexual behaviour by reducing the duration of contact of sexually hypoactive bucks with sexually hyperactive bucks

Sexual stimulation of bucks by a photoperiodic treatment

The bucks were 5 years old at the beginning of the study. On 1 November, bucks were allocated into two groups with similar body and testicular weights. Bucks from one group ($n = 13$) were subjected to natural day length throughout the study; this was the sexually hypoactive group (BW: 51 ± 5 kg; testicular weight: 130 ± 8 g). Bucks from the other group ($n = 6$) were subjected to artificial long days as described in Experiment 1; this was the sexually hyperactive group (BW: 54 ± 3 kg; testicular weight: 125 ± 10 g).

Experimental design

On 29 March, 13 sexually hypoactive bucks were allocated into two groups with similar body and testicular weights. On 30 March (Day 0), one group of bucks was joined with two sexually hyperactive bucks for 31 days; this was the contact group ($n = 6$; BW: 73 ± 3 kg; 121 ± 3 g). The other group of males was joined with two sexually hyperactive males for 10 days and then withdraw from them until the end of the study; this was the withdraw group ($n = 7$; BW: 74 ± 3 kg; testicular weight 121 ± 9 g).

Measurements

Plasma testosterone concentrations were determined on days 2 and 0 just before introducing the bucks, and then on day 1 and every 3 days until 31 days after introducing the bucks. Sensitivity of assay was 0.15 ng/mL, and the intra-assay CV was 9.1%. Sexual behaviour displayed by bucks of the contact and withdraw groups was determined for 10 min after introducing the bucks, and then every 7 days throughout the study. Testosterone and sexual behaviour were determined as described in Experiment 1.

Statistical analysis

In both experiments, the mean data of testosterone concentrations (which was the “experimental unit”) were analysed using a two-way ANOVA with repeated measurements (group and time of study) followed by the independent Student's *t*-test for individual point comparisons when there were significant interactions. In both Experiments, the total number of nudging events (which was the “experimental unit”) displayed by sexually hyperactive or sexually hypoactive bucks were analysed for a time effect by a Chi-square test. In addition, the total number of nudging events displayed by the sexually hypoactive bucks was compared using a Chi-square test for goodness of fit with a random distribution of 50% in each group as the null hypothesis. Analyses were computed using the [System Statistics Package \(2009\)](#). Data are expressed as the mean \pm SEM, and differences were considered significant at the level of $P \leq 0.05$.

Results

Experiment 1

Plasma testosterone concentrations

Testosterone concentrations varied over time of study ($P < 0.01$), and there was an effect of group ($P < 0.05$) and an interaction between these two factors ($P < 0.05$). Testosterone concentrations were low (< 5 ng/mL) and did not differ between groups before the introduction of bucks ($P > 0.05$). Thereafter, on day 1, testosterone concentrations increased in both groups and did not differ between them ($P > 0.05$). Then, in males from separated

group, testosterone concentrations decreased progressively from 10 to 30 days, whereas in males from the contact group, these concentrations increased until 20 days. The testosterone plasma concentrations were greater in males of the contact group than in those of the separated group at 20 and 30 days and did not differ afterwards ($P < 0.01$; [Fig. 1](#)).

Sexual behaviour

The total number of nudging events varied over the time ($P < 0.01$), and males from the contact group displayed more nudging than males from the separated group from 0 to 30 days and did not differ afterwards ($P < 0.001$; [Fig. 2](#)).

Experiment 2

Plasma testosterone concentrations

Testosterone concentrations varied over time of study ($P < 0.001$), and there was an effect of group ($P < 0.05$) and an interaction between these two factors ($P < 0.001$). Testosterone concentrations were low (< 5 ng/mL) and did not differ between groups before the introduction of bucks ($P > 0.05$). Thereafter, on day 1, testosterone concentrations increased dramatically in both groups, reaching maximum concentrations 4 days after introduction of the sexually hyperactive bucks without differences between groups ($P > 0.05$). Afterwards, in males from the withdraw group, testosterone concentrations decreased progressively, reaching low concentrations (< 5 ng/mL) as soon as 22 days and remained low until the end of study. In contrast, in the contact group, these concentrations remained elevated and were greater than in those from the withdraw group from 19 to 31 d after introduction of the sexually hyperactive males ($P < 0.05$; [Fig. 3](#)).

Sexual behaviour

The total number of nudging events displayed by the bucks from the contact and withdraw groups varied over time ($P < 0.05$). The number of nudging displayed by both groups did not differ immediately after joined with the sexually hyperactive males ($P > 0.05$), but bucks from the withdraw group displayed more nudging than the contact group 7 days after the introduction of the sexually hyperactive bucks ($P < 0.05$; [Fig. 4](#)). Afterwards, bucks from the contact group displayed more nudging than those from the withdraw group 14, 21 and 28 days after the introduction of the sexually hyperactive bucks.

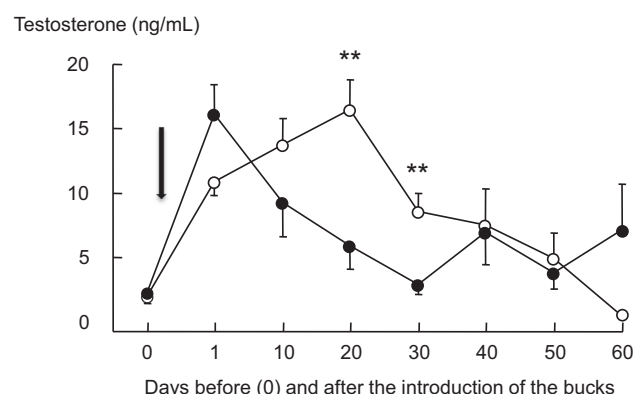


Fig. 1. Plasma testosterone concentration (mean \pm SEM) in sexually hypoactive bucks ($n = 6$) joined with sexually hyperactive bucks for 60 days ($n = 2$; ○) or separated 1.5 m from these bucks ($n = 6$; ●). Bucks were rendered sexually hyperactive by exposure to 2 months of long days (16 h of light per day) from 15 November followed by natural photoperiod conditions. Significant differences are denoted with ** ($P < 0.01$). ↓ Indicates the moment of introduction of the bucks.

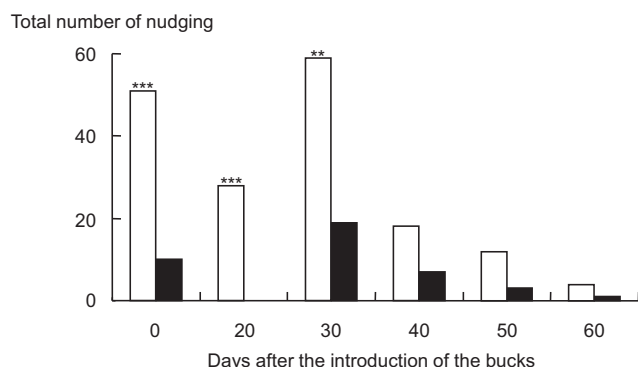


Fig. 2. Total number of nudging events displayed by sexually hypoactive bucks ($n = 6$) joined with sexually hyperactive bucks for 60 days ($n = 2$; □) or separated 1.5 m from these bucks ($n = 6$; ■). Bucks were rendered sexually hyperactive by exposure to 2 months of long days (16 h of light per day) from 15 November followed by natural photoperiod conditions. Significant differences are denoted with ** ($P < 0.01$) and *** ($P < 0.001$).

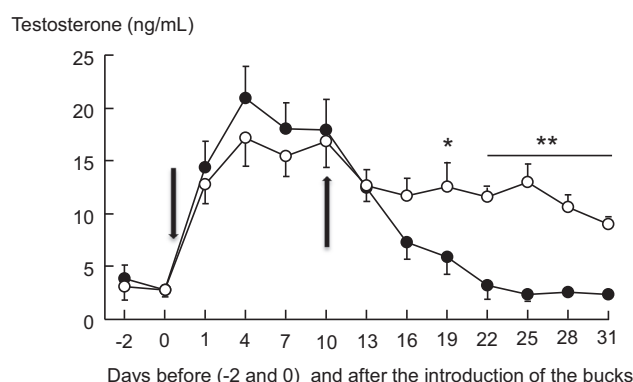


Fig. 3. Plasma testosterone concentrations (mean \pm SEM) in sexually hypoactive bucks ($n = 6$) joined with sexually hyperactive bucks for 31 days ($n = 2$; ○) or 10 days and then separated from the sexually hyperactive bucks ($n = 7$; ●). Bucks were rendered sexually hyperactive by exposure to 2 months of long days (16 h of light per day) from 15 November followed by natural photoperiod conditions. Significant differences are denoted with * ($P < 0.05$) and ** ($P < 0.01$). ↓ Indicates the moment of introduction of the bucks. ↑ Indicates the moment when males were removed from the 10-day separated group.

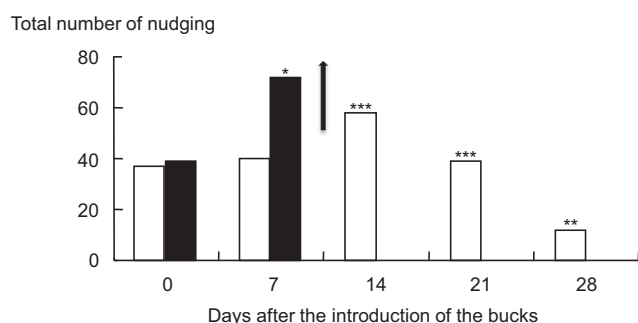


Fig. 4. Total number of nudging events displayed by sexually hypoactive bucks ($n = 6$) joined with sexually hyperactive bucks for 31 days ($n = 2$; □) or 10 days and then separated from the sexually hyperactive bucks ($n = 7$; ■). Bucks were rendered sexually hyperactive by exposure to 2 months of long days (16 h of light per day) from 15 November followed by natural photoperiod conditions. Significant differences are denoted with * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$). ↑ Indicates the moment when males were removed from the 10-day separated group.

Discussion

The findings of the present experiments support our hypothesis that physical separation and reduced duration of contact with sexually hyperactive bucks reduce the testosterone plasma concentrations and sexual behaviour of bucks in sexual rest when exposed to the “buck-to-buck effect”. Indeed, in experiments 1 and 2, testosterone plasma concentrations and sexual behaviour increased at the introduction of the sexually hyperactive bucks, but afterwards, both variables decreased in separated and reduced duration of contact groups. Altogether, these findings clearly indicate that in sexually hypoactive bucks exposed to the “buck-to-buck effect”, the permanent physical contact, and the long-term duration of contact with the sexually hyperactive bucks are necessary to maintain high testosterone plasma concentrations and intense sexual behaviour.

The results of experiment 1 clearly showed that the physical separation from the sexually hyperactive bucks decreased the testosterone plasma concentrations in sexually hypoactive bucks. Indeed, 1 day after the introduction of the sexually hyperactive males, testosterone level increased dramatically in separated bucks as in the same way as in the males of the contact group. However, testosterone concentration subsequently decreased progressively in bucks of the separated group and were lower than those in males of the contact group 20 and 30 days after the introduction of sexually hyperactive males. Interestingly, despite the pattern of testosterone secretion in bucks from the separated group, their sexual behaviour was lower than that displayed by bucks from the contact group in the first 30 days after joining with the sexually hyperactive bucks. This indicated that the transient increase in testosterone, the hormone responsible for the sexual behaviour of males (Signoret et al., 1982) was not sufficient to stimulate the sexual behaviour in the separated group, as it was the case in bucks of the contact group. Therefore, in the present study, it is likely that in the separated group, the exteroceptive signals emitted by the sexually hyperactive bucks when interacting freely with the sexually hypoactive males (i.e., odor, vocalizations and/or the free sexual interactions displayed by bucks) stimulated testosterone secretion at the initial introduction of the sexually hyperactive bucks, but thereafter, were unable to maintain high testosterone concentrations. This hypothesis is supported by the fact that in seasonal anestrus goats, the exteroceptive signals emitted by bucks stimulate the hypothalamo-pituitary-gonad axis. Indeed, exposure to the odor of bucks' hair or live buck vocalizations stimulated the activity of GnRH neurons, LH secretion, estrous behaviour and/or ovulations (Claus et al., 1990; Delgadillo et al., 2012; Sakamoto et al., 2013). The findings from our separated group are consistent with those described in goats and ewes, in which ovulatory response is lower in females separated from males by a wire fence or a passage than in those in full contact with them (Shelton, 1980; Chemineau, 1987; Pearce and Oldham, 1988). Taken together, our findings indicate that in sexually hypoactive bucks, direct and physical permanent contact with sexually hyperactive bucks is necessary to obtain the full endocrine and sexual responses of males exposed to the “buck-to-buck effect”, as already described in female goats and ewes.

The results of experiment 2 clearly showed that the reduction of duration of contact with the sexually hyperactive bucks decreased testosterone plasma concentration and sexual behaviour. In this experiment, as in experiment 1, testosterone increased dramatically after the introduction of the sexually hyperactive bucks, and concentrations did not differ between bucks from the contact and the withdraw groups in the first 16 days. This lack of difference was probably due to the intense sexual behaviour displayed by the photoperiodic-treated, sexually hyperactive bucks, as reported recently in bucks exposed to the “buck-to-buck effect”

(Delgadillo et al., 2022). Afterwards, testosterone concentration decreased progressively in the withdraw group from 13 to 31 days after joining with the sexually hyperactive males, and these concentrations were significantly lower than those registered in the contact group. It is interesting to note that in the withdraw group, sexually hyperactive bucks were removed 10 days after their introduction, and since they were removed, testosterone concentrations began to decrease until the end of the study. These findings indicate that the continuous presence of the sexually hyperactive bucks is necessary to prevent the decrease of testosterone concentrations, as occurred in the contact group. Interestingly, the sexual behaviour of the bucks from the withdraw group was significantly lower than that displayed by the bucks from the contact group from 14 to 28 days. This difference in sexual behaviour was most likely due to the decrease in testosterone, as previously reported at the end of breeding season in bucks kept under natural photoperiod conditions or in those exposed to photoperiodic treatments (Delgadillo et al., 1999; Zarazaga et al., 2019). Taken together, these findings indicated that the permanent presence of the sexually hyperactive bucks allowed to stimulate the hypothalamo-pituitary-gonad axis and the sexual behaviour of sexually hypoactive bucks. These findings are consistent with those reported in female goats exposed to the male effect using sexually hyperactive bucks. Indeed, the intermittent contact of female goats with bucks increased the frequency of LH pulses at the introduction of the male but decreased when the males were removed (Bedos et al., 2014). Moreover, the permanent presence of the sexually hyperactive bucks allowed goats to ovulate during the natural seasonal anestrus. However, when sexually hyperactive bucks were removed from the group of goats, ovulations immediately stopped (Delgadillo et al., 2015). Finally, the permanent presence of the sexually hyperactive bucks maintained high LH plasma concentrations during the seasonal anestrus in ovariectomized goats bearing subcutaneous implants containing estradiol (Muñoz et al., 2017). Taken together, these findings indicate that the neuroendocrine response of bucks exposed to the “buck-to-buck effect” is similar to that observed in goats exposed to the male effect when sexually hyperactive males were used.

Conclusion

Our results show that the physical separation and reduced duration of contact with the sexually hyperactive bucks decreased testosterone plasma concentrations and sexual behaviour of bucks in sexual rest exposed to “buck to buck effect”. To the best of our knowledge, these are the first results indicating some necessary conditions to successfully stimulate the neuroendocrine and sexual activities of male goats in a sustainable manner through sociosexual interactions.

Ethics approval

The collegiate body of investigation at the University Antonio Narro approved all the procedures performed in these studies (reference: 35-38111-425501002-2787). Also, the experimental procedures were in accordance with the official Mexican rules governing the technical specifications for the production, care, and use of laboratory animals (*Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio*, 2001).

Data and model availability statement

None of the data were deposited in an official repository but are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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