Rams sexually activated in spring by light treatment stimulate social and sexual activities in non-activated rams

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ABSTRACT

Twelve rams were used to confirm whether rams sexually activated in spring by light treatment increase social and sexual activities of non-treated rams in the presence of ewes. Males were divided into 2 groups: light-treated rams (group L, n = 6), which were exposed to artificial long days (16 h light) for 2 months (1 Nov-31 Dec), or non-exposed, control rams (group C, n = 6), exposed to the natural photoperiod. At the end of the long-day period, light-treated rams were returned to the natural photoperiod, groups isolated from each other. In mid-Feb, 3 rams from each group were put together such that 4 experimental groups were created: the remaining light-treated rams (L, n = 3), the remaining control rams (C, n = 3), and the other treated (L-mixed n = 3) and non-treated (C-mixed, n = 3) rams, exposed to 30, 30, and 60 ewes, respectively, in 3 separate paddocks. To identify each ram, large (30-cm-high) numbers were painted on the sides and rump of the males with washable sheep spray paint. Behaviors were documented by 4 video cameras using 6 30-minute segments from 1600 to 1800 h the following day (27 hours). C rams presented the lowest proportion of activities ($P < 0.01$), and C-mixed rams exhibited proportions similar to L and L-mixed rams. C rams exhibited the fewest fight attempts ($P < 0.001$), fighting ($P < 0.05$), pushing ($P < 0.001$), and sniffing another ram. The groups differed significantly ($P < 0.001$) in pushing, pawing, flehmen, and attempt mounting, and C rams had the fewest interactions. In conclusion, rams exposed to 2 months of long days at late-autumn early-winter increased social and sexual activities of unexposed rams. These observations can be explained by a "ram-to-ram effect", which can enhance the endocrine activity of rams in sexual rest if they are joined with rams who have been made sexually active by a photoperiodic treatment. These findings suggest that socio-sexual interactions could be used to restrict out-of-season reproduction in this species.

Introduction

In recent years, "biostimulation" (i.e., the stimulatory effects on the reproductive characteristics of females; e.g., the onset of puberty, estrous expression, and ovulation induction, which are induced by the presence of a male) in lieu of exogenous hormones and drugs has been advocated as a means of controlling and improving the productivity of sheep and goats (Martin and Kadokawa, 2006). The avoidance of exogenous hormones and drugs might be a long-term phenomenon and, therefore, it makes sense for animal producers everywhere to move towards clean, green, and ethical practices. The year-round presence of sexually active bucks that have been stimulated by 2 months of exposure to an extra-light

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treatment in autumn and winter can prevent seasonal anestrus in goats (Delgadillo et al., 2015). If those bucks were removed in the natural season of anovulation, females stopped cycling and entered anestrus, immediately. In sheep, the presence of rams that have been sexually activated in spring by exposure to long days for 2 months prolongs the ovarian and estrous activity of Rasa Aragonesa ewes in spring, effectively suppressing their seasonal anestrus (Abecia et al., 2015), advances the reactivation of postpartum sexual activity in ewes in the middle of seasonal anestrus, when they lamb at the end of the reproductive season (Abecia et al., 2017), or induces puberty in autumn-born ewe-lambs (Abecia et al., 2016). In addition, if used for a ram effect, sexually activated rams significantly increase the proportion of sheep that become pregnant and the number of lambs born per ewe in May (Abecia et al., 2017). The mechanisms involved in the induction of ovarian activity in spring are mediated through changes in LH secretion because light-treated sexually activated rams induce LH preovulatory surges in ewes in the seasonal anestrus (Abecia et al., 2019), and the continuous presence of sexually active rams prevents the seasonal decrease in plasma LH concentrations in OVX + E ewes, which prevents the seasonal negative feedback of estradiol on LH secretion (Abecia et al., 2020).

Recently, we demonstrated that endocrine activity in bucks (Delgadillo et al., 2022) and rams (Abecia et al., 2022) in sexual rest can be stimulated if they are exposed to sexually stimulated bucks and rams, respectively, which we called "buck-to-buck" and "ram-to-ram" effects, respectively. Furthermore, the bucks that had been exposed to the buck-to-buck effect were, thereafter, able to induce sexual and reproductive activities of seasonal anestrous in goats by the "classical" phenomenon called the male effect (Delgadillo et al., 2022). In both species, the presence of males that had been sexually activated by light treatment induced an increase in LH and testosterone in the stimulated bucks and rams.

The aim of this study was to investigate whether rams that had been sexually activated in spring by light treatment improved the social and sexual activity of non-treated rams once they were exposed to ewes.

Material and methods

Animals

Twelve meat mixed-breed rams (Merino x Berrichon x Castellana) were used. Males were kept in a shaded, open pen under natural photoperiod before being assigned to 1 of 2 groups: light-treated rams (group L, n = 6), which were induced into a sexually active state by exposure to artificial long days (16 h of light/d) for 2 months (1 Nov to 31 Dec), and non-exposed, control rams (group C, n = 6), which were exposed to the natural photoperiod. For the treated group, artificial light was provided in the morning (0600 h to 0900 h) and in the evening (1600 h to 2200 h), which was controlled by an electronic timer, and light intensity was >300 lux at animal eye-level (Chemineau et al., 1992). At the end of the long-day period (31 Dec), light-treated rams were returned to natural photoperiod conditions, although both groups were kept isolated from each other.

In mid-Feb, 3 rams from each group were put together such that 4 experimental groups were created: the remaining light-treated rams (L, n = 3), the remaining control rams (C, n = 3), and a mixed set that had treated (L-mixed, n = 3) and non-treated (C-mixed, n = 3) rams, which were exposed to 30, 30, and 60 ewes, respectively, in 3 separate paddocks. To identify each ram, large (30-cm-high) numbers were painted on the sides and rump of the males with washable sheep spray paint. Ewes were not hormone- or light-treated.

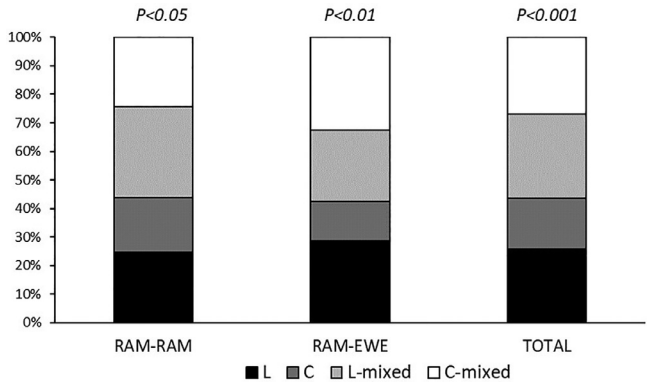

Figure 1. Proportions (%) of ram-ram, ram-ewe, and overall activities of light-treated rams (L, n = 3), non-treated control rams (C, n = 3), and a mixed group that had three treated (L-mixed, n = 3) or three non-treated (C-mixed, n = 3) rams. Light-treated rams had been induced into a sexually active state by exposure to artificial long days (16 h of light/d) for two months (1 Nov to 31 Dec). In mid-Feb, three rams from each group were put together (L-mixed and C-mixed).

Behavioral records

Activities were documented by 4 video cameras (Samsung SDC-9443BC) that recorded 6 30-min segments from 1600 h (hour 0), when rams had their first contact with ewes, until 1800 h the following day (hour 27). For each ram, 2 classes of activities were recorded: ram-ram activities (fight attempt, fighting, pushing, touching, ram sniffing, bullying, ram mounting, and pawing), and ram-ewe activities (chasing ewes, pushing ewes, anogenital sniffing, urine sniffing, pawing, flehmen, approaches, attempt mounting, and mounting).

Statistical analysis

The IBM SPSS Statistics program for Windows (26.0) was used to analyze the data. Normality was assessed by the Kolmogorov–Smirnov Test. The X^2 test was used to detect statistically significant differences among groups in the distribution of the frequency of each activity recorded.

Results

In the experiment, most (68%) of the interactions were between rams, rather than ram-ewe (32%), although the frequency of activities differed significantly among groups in ram-ram ($P < 0.05$), ram-ewe ($P < 0.01$), and overall activity ($P < 0.001$) (Figure 1). C rams exhibited significantly ($P < 0.01$) fewer interactions than did the other 3 groups, and L, C-mixed, and L-mixed rams exhibited similar proportions of activities.

Anogenital and urine sniffing, and chasing ewes were the most common (57%) interactions exhibited by rams (Figure 2). Touching, bullying, and fighting other rams were the most common ram-ram interactions (Figure 3), and anogenital sniffing was the most common ram-ewe interaction (Figure 4). The distributions of ram-ram (Figure 5) and ram-ewe (Figure 6) activities differed significantly among groups. With the exception of ram and pawing mounting, the distribution of ram-ram interaction differed among groups, and the C rams exhibited the fewest fight attempts ($P < 0.001$), fighting ($P < 0.05$), pushing ($P < 0.001$), and sniffing another rams. C and L-mixed rams had the fewest touching rams ($P < 0.001$), and L and C rams had the fewest bullying ($P < 0.001$) activities (Figure 5). Among ram-ewe activities, groups differed significantly ($P < 0.001$) in the frequencies of pushing, pawing, flehmen, and attempt mounting, and the C groups exhibited the fewest activities.

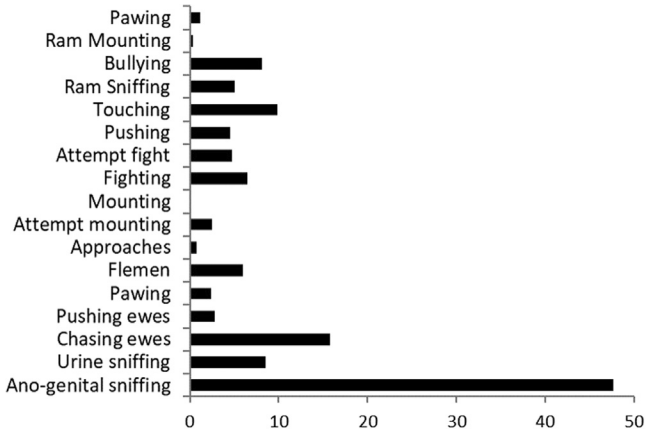

Figure 2. Proportion (%) of ram-ram activities, and ram-ewe activities, exhibited by rams, documented by four video cameras that recorded six 30-minute segments from 1600 h (hour 0), when rams had their first contact with ewes, until 1800 h the following day (hour 27).

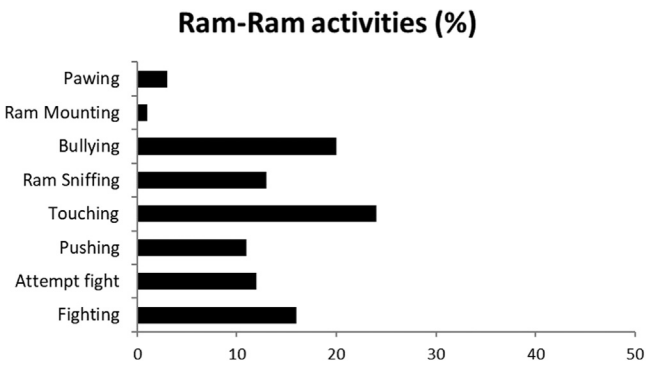

Figure 3. Proportion (%) of ram-ram activities exhibited by rams documented by four video cameras that recorded six 30-minute segments from 1600 h (hour 0), when rams had their first contact with ewes, until 1800 h the following day (hour 27).

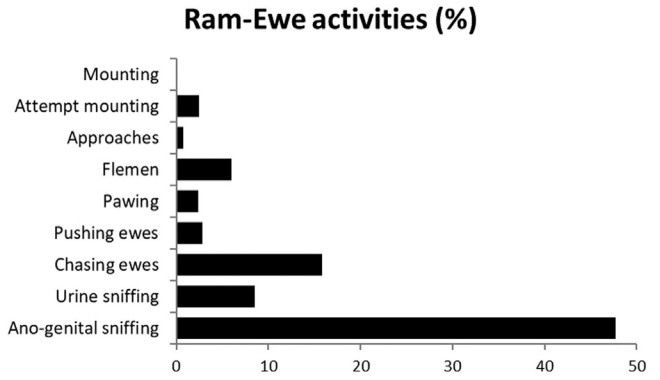

Figure 4. Proportion (%) of ram-ewe activities exhibited by rams based on data recorded by four video cameras that recorded six 30-minute segments from 1600 h (hour 0), when rams had their first contact with ewes, until 1800 h the next day (hour 27).

Discussion

Differences in the behavior of the L and C rams indicated that the light treatment was effective in inducing an intense sexual activity in treated rams in spring. Rams that had been induced into a sexually active state by exposure to 2 months of long days influenced the sexual activity of non-exposed rams within a short time because the latter exhibited behaviors within the first 24 h together, that was similar to that of the treated rams, and even better than their control mates, which remained isolated with the ewes.

Our research has demonstrated an acute increase of plasma LH and testosterone concentrations in rams in sexual rest after the introduction of sexually activated rams (Abecia et al., 2022). The short-time increase in both hormones was evident within the first 4 h after the introduction of the stimulating rams and persisted 24 h afterward. In addition, we have documented that type of stimulation of endocrine activity in the males by the presence of light-treated bucks (Delgadillo et al., 2022). In this species, the presence of stimulated bucks induced sexual and reproductive activities in seasonally anestrous goats, which was similar to that of the male effect, such that stimulated rams induced ovulation in goats later. In our experiment, probably, the L rams induced the same response in the C-mixed rams such that the latter showed activity similar to that of the light-treated rams, which was significantly different than that of the C rams, which were kept isolated from the L rams.

Maina and Katz (1997) found that interactions with previously mated males enhanced the sexual performance of rams and sug-

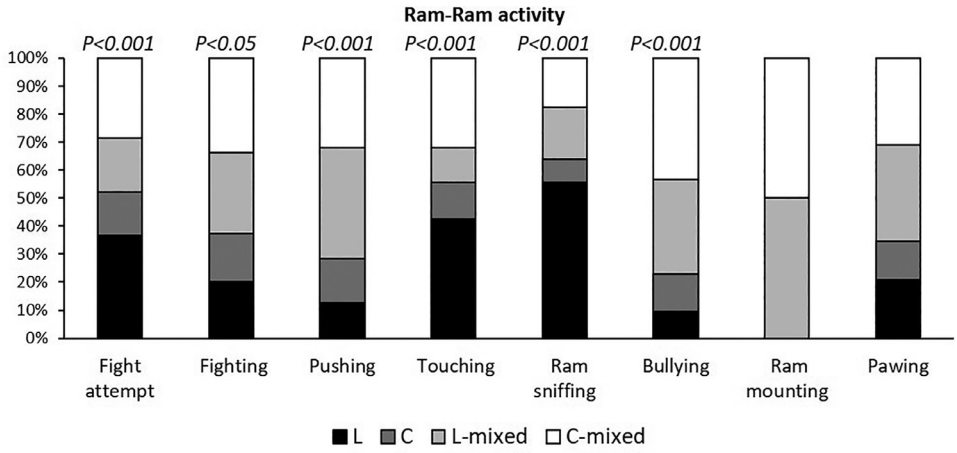

Figure 5. Proportion (%) of ram-ram activities of light-treated rams (L, n = 3), non-treated control rams (C, n = 3), and two mixed groups that contained three treated (L-mixed, n = 3) and three non-treated (C-mixed, n = 3) rams. Light-treated rams had been induced into a sexually active state by exposure to artificial long days (16 h of light/d) for two months (1 Nov to 31 Dec). In mid-Feb, three rams from each group were put together (L-mixed and C-mixed).

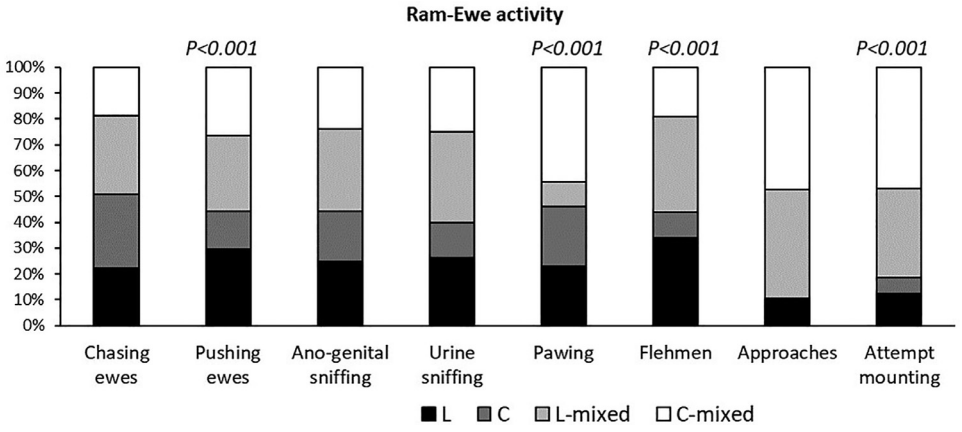

Figure 6. Proportion (%) of ram-ewe activities of light-treated rams (L, n = 3), non-treated control rams (C, n = 3), and two mixed groups that had three treated (L-mixed, n = 3) and three non-treated (C-mixed, n = 3) rams. Light-treated rams had been induced into a sexually active state by exposure to artificial long days (16 h of light/d) for two months (1 Nov to 31 Dec). In mid-Feb, three rams from each group were put together (L-mixed and C-mixed).

gested that the cues involved might be the scent of the ewe, a scent associated with the actual mating activity, or some behavioral cue displayed by the stimulus ram. Price et al. (1991), however, found that the sexual performance of mature rams was not enhanced by exposure to a copulating ram. Furthermore, observing the mounting behavior of other males that were competing for females did not increase sexual performance compared to that of rams that had observed a group of males alone in a pen or an empty pen (Price et al., 2001). In addition, direct competition for ewes in estrus failed to increase the sexual performance of rams in subsequent serving-capacity tests. Apparently, the high sexual activity exhibited by control rams that had joined light-treated rams in our experiment was not caused by observations of light-activated rams copulating (no matings were recorded), or by interactions with ewes. That said, if rams respond to exposure to estrous females by exhibiting a significant increase in LH pulse frequency, and in basal and mean LH and testosterone levels (Gonzalez et al., 1988), and increases in LH and testosterone concentrations can be detected within the first 4 days if rams are in continuous contact with estrual ewes (Ungerfeld and Silva, 2004), it can be argued that the introduction of ewes in the groups of rams might have caused the increase in the sexual activity of rams. In our experiment, however, the ewes were not synchronized into estrus by any hormone or light treatment, and the experiment occurred in the seasonal anestrus period; therefore, presumably, few ewes could have exhibited estrus when they were housed with the rams. Therefore, we suggest that neither the mating performance of other rams nor the presence of ewes influenced the response of C-mixed rams.

Conclusion

In conclusion, rams that had been exposed to 2 months of long days at the end of winter increased the social and sexual activities of unexposed rams 40 days after the former were returned to the natural photoperiod. These observations can be explained by the "ram-to-ram effect" described by our group, which can enhance the endocrine activity of rams in sexual rest if they are joined with rams who have been made sexually active by a photoperiodic treatment. These findings suggest that socio-sexual interactions could be used to restrict out-of-season reproduction in this species.

Authors' contribution

The idea for the study was conceived by C.P. and J.A.A. The experiments were designed by C.P., J.A.D., P.C., M.K. and J.A.A. and performed by C.P., A.A.L., and S.A.C. The data were analyzed by C.P., J.A.A., A.A.L., and the manuscript was written by C.P., J.A.A., J.A.D., P. C.. All authors reviewed and approved the final version of the manuscript.

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Ethical Considerations Statement

The study involved an organic sheep flock in Salamanca (Spain). The Ethics Committee for Animal Experiments at the University of Zaragoza approved all of the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Conflict of Interest

The authors declare no conflict of interest.

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