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Assessment of sexual activity in rams stimulated with artificial light and its impact on unstimulated rams during the initial 14 days of male effect

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ABSTRACT

Seasonal anoestrous reduces reproductive efficiency in sheep in temperate regions. Artificial photoperiod manipulation is a non-hormonal approach that stimulates ram sexual activity and enhances the "ram effect" on ewes. This study evaluated the sexual behaviour of rams subjected to artificial long-day photoperiods (L group) and their effect on non-stimulated rams (C group) during the first 14 days after introduction to anoestrous ewes. Eleven rams were assigned to photostimulated ($n = 6$) or control ($n = 5$) treatments, forming four groups: L, C, L+C (stimulated rams exposed to controls), and C+L (controls exposed to stimulated rams). Continuous video recording documented 5707 behavioural events. Photostimulated rams (L) exhibited higher activity (mean = 747.7 behaviours/ram) than other groups (C+L: 519.5; L+C: 443.0; C: 365.3; $p < 0.01$), with anogenital sniffing, approaching and chasing ewes being the most frequent behaviours. Activity peaked on days 2–5 post-introduction, mainly in the afternoon. Control rams housed with stimulated rams were more active than isolated controls, suggesting a social facilitation effect. These findings confirm that photoperiod manipulation enhances ram sexual activity and may influence non-stimulated rams through social interaction, as the different groups of animals exhibited distinct types and amounts of activity.

1. Introduction

Rams from temperate-adapted breeds experience seasonal reproductive changes, including testicular regression and altered hormonal profiles during the non-breeding season, which reduce libido (Milczewski et al., 2015). This limitation can be mitigated through artificial photoperiodic stimulation (Arrebola et al., 2022). Rams exposed to artificial long-day lighting during deep anoestrous not only exhibit increased reproductive activity but can also stimulate LH secretion in non-stimulated rams, thereby enhancing sexual behaviour and mating likelihood (José A Abecia et al., 2020). Furthermore, light-stimulated rams show greater social and sexual activity than non-stimulated rams during the first 27 h following ewe introduction (Palacios et al., 2023).

Sheep are seasonally breeders whose reproductive activity is mainly regulated by photoperiod (Kopycińska et al., 2022). In temperate regions, the transition between breeding and non-breeding seasons is accompanied by marked neuroendocrine changes that affect both sexes, although the reproductive status of rams is of particular importance for flock fertility. During the non-breeding season, males show a reduction in testicular size, testosterone secretion, libido, and sperm production (Chemineau et al., 2008; Lincoln and Short, 1980).

In rams, photoperiod acts directly on the hypothalamic-pituitary-gonadal axis. Long days suppress gonadotrophin-releasing hormone (GnRH) and luteinising hormone (LH) secretion, while exposure to decreasing day length restores testicular function and sexual activity (Lincoln and Peet, 1977). Manipulation of this environmental cue through artificial light programmes that mimic short-day conditions can therefore be used to induce or extend the breeding season in males (Chemineau et al., 1992; Delgadillo et al., 2002)

Additionally, sexually active rams influence the reproductive activity of ewes through the so-called "ram effect," a neuroendocrine response triggered by the introduction of novel or sexually active males that induces ovulation in anoestrous females. Understanding how light-stimulated rams interact with non-stimulated ones, and how this affects overall sexual behaviour and flock synchronisation, is therefore essential for improving natural breeding management without relying on hormonal treatments (A Chanvallon et al., 2011; Oldham and Martin,

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1978).

The introduction of a sexually active ram to seasonally anoestrous ewes increases LH pulse frequency in most females, triggering ovulation- the "ram effect" (José A. Abecia et al., 2020; Gelez and Fabre-Nys, 2004). This response is usually preceded by a sustained plasma oestradiol increase lasting 8–56 h, although some ewes respond within 0–8 h (Fabre-Nys et al., 2016). Breed influences the timing of the response; for instance, Highlander ewes respond earlier than Suffolk ewes (Cox et al., 2015). Ovulation rates tend to be higher towards the end of anoestrous (Chanvallon et al., 2011).

The collection of reproductive and behavioural data has evolved from invasive methods, which may induce stress and affect results (Saldaña–Ríos et al., 2016), to non-invasive technologies such as GPS tracking (Bouten et al., 2013) and automated oestrus detection systems (Odintsov Vaintrub et al., 2021). Video recording, particularly when combined with automated analysis, represents a promising approach for monitoring reproductive behaviour (Odagiri et al., 1995); however, it generates very large datasets, manual annotation is labour-intensive and prone to fatigue-related errors, and the performance of automated models depends critically on high-quality, well-labelled training data (Siegford et al., 2023).

The present study aimed to evaluate the reproductive activity of rams subjected to artificial photoperiodic stimulation during seasonal anoestrous and determine whether their presence could elicit a sympathetic reproductive behaviour in non-stimulated rams. We hypothesised that photostimulated rams would show greater reproductive activity and that non-stimulated rams housed nearby would display a sympathetic response within 14 days of the “ram effect”.

2. Material and methods

2.1. Animals and experimental design

The study was conducted on an extensive sheep farm in the province of Salamanca, Spain (41°01’04’’N, 5°46’40’’W, 800 m.a.s.l.), in the northwest of the Iberian Peninsula. Eleven crossbred meat rams (Merino × Berrichon × Castellana), aged 3–4 years and weighing 80–105 kg, were used. The relatively small number of animals reflects the pilot nature of this study, with plans to increase the sample size in future research. Initially, all rams were housed in an outdoor shaded pen under natural photoperiod conditions, before being randomly assigned to two groups: light-stimulated rams (group L, $n = 6$) and non-stimulated controls (group C, $n = 5$). Rams in group L were exposed to artificial long days for two months (1 November-31 December), consisting of natural daylight (08:00–18:00 h) supplemented with artificial light (18:00–24:00 h; >300 lux at eye level, controlled by an electronic timer). After this period, light-treated rams were returned to natural photoperiod conditions for one month and kept completely isolated from the controls. Group C rams remained under natural photoperiod conditions throughout.

In mid-February, after the stimulation period, rams were reorganised to allow interaction between individuals from both initial groups, forming four experimental groups housed in three separate pens with 120 untreated ewes (Merino × Berrichon × Castellana). The ewes, aged 2–5 years and weighing 60–80 kg, had last been exposed to rams at the end of the natural breeding season in October and included both recently lambed and non-pregnant animals. The groups were: 1) three light-stimulated rams (L, $n = 3$) with 30 ewes; 2) three control rams (C, $n = 3$) with 30 ewes; 3) a mixed pen containing the remaining three L rams and two C rams with 60 ewes.

To assess whether the presence of one type of ram (L or C) influenced the sexual behaviour of the other, two subgroups were defined within the mixed pen: light-stimulated rams influenced by control rams (L+C, $n = 3$), and control rams influenced by light-stimulated rams (C+L, $n = 2$). This resulted in four experimental groups: L = 3; C = 3; L+C = 3; C+L = 2.

The observation period covered 14 days, corresponding to the early phase of the male effect, when behavioural responses are typically most pronounced. This duration was selected to focus on the male-male behavioural dynamics before any influence of female oestrous could occur. In these latitudes, fertile oestrous activity in ewes following the ram effect usually begins between days 18 and 21 after male introduction (Abecia et al., 2006). Therefore, a conservative safety margin of four days was applied to ensure that the study strictly assessed the male-male interactions without interference from female behavioural responses.

All procedures were approved by the Animal Experimentation Ethics Committee of the University of Salamanca. Animal care complied with Spanish legislation (RD1201/05) and the European Union Directive 2010/63 on the protection of animals used for scientific purposes.

2.2. Behavioural recording procedure and visualization

Sexual activities were recorded using four Samsung SDC-9443BC video cameras (PC Componentes y Multimedia SLU, Murcia, Spain). A total of 45 video segments of 30 min each were recorded between 20:00 h on February 12 (Day 0 post-stimulation) and 08:00 h on February 26 (Day 14 post-stimulation). Recordings were distributed across three time periods according to season and location: 1) morning (08:30–13:30 h); 2) afternoon (13:30–19:30 h); 3) night (19:30–8:30 h).

Due to technical malfunctions in the recording equipment, behavioural data from days 6, 7, 10, and 11 post-stimulation were unavailable, reducing the analysis to 11 days of observation. As these days corresponded to the metaestrus and dioestrus phases, when ewes are not receptive, their absence is unlikely to affect the study’s replicability. Furthermore, they fall outside the peak period of male sexual activity (3–4 days after ram introduction), and no significant differences in sexual behaviour were detected, indicating that the remaining recordings are sufficient for robust analysis.

Each video segment was viewed by a single observer, who compiled a behavioural database. Rams were identified in the recordings by unique morphological characteristics, such as coat colour, body size, tail length, ear shape, and facial profile. For each individual, nine sexual behaviours were recorded and quantified: anogenital sniffing (AGS), urine sniffing (URS), flehmen (FLE), chasing ewes (CHE), pushing ewes (PUE), pawing ewes (PAE), approaching ewes (APE), mount attempt (MOA), and mount mating (MOM).

For each observation of these behaviours, the date and time of occurrence were recorded, as well as the experimental group (L, C, L+C, or C+L) to which the ram belonged.

2.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics software for iOS, version 26.0 (IBM, Armonk, NY, USA). To ensure a unique entry for each behaviour displayed by an individual ram at a specific time, the variable "sexual activity" was encoded with values from 1 to 9, corresponding to the nine observed behaviours. The variables "day", "time period", and "experimental group" were also coded accordingly. This produced a qualitative frequency database of categorical variables, totalling 5707 records.

To evaluate the independence or association between these categorical variables, the Chi-square test (χ^2), was applied, assuming an equal distribution of expected frequencies. Statistical significance was assessed at a 95 % confidence level ($\alpha = 0.05$). Results were normalised and expressed as the average number of observations per ram.

To examine differences among sexual activities, data were grouped to calculate the mean number of observations for each of the nine behavioural variables. This transformed the database from a single qualitative variable ("sexual activity") into nine behavioural variables. Normality was tested using the Kolmogorov-Smirnov test, which indicated a clear deviation from normality, leading to rejection of the null hypothesis.

As a result, the non-parametric Kruskal-Wallis H test was used to identify significant differences in sexual activity among experimental groups (observations per ram). Homogeneous subsets were identified using Bonferroni correction with the same confidence level ($\alpha = 0.05$) as for the Chi-square test. The influence of several fixed factors was analyzed, specifically: experimental group, recording day, and time of day.

3. Results and discussion

3.1. Frequency patterns of sexual activity

Across the study period, light-stimulated rams (L) displayed significantly greater sexual activity than all other groups ($p < 0.01$), averaging 747.7 behaviours per ram, compared with 519.5 in C+L, 443.0 in L+C, and 365.3 in C. These findings reinforce previous evidence that photoperiod manipulation enhances male reproductive behaviour during seasonal anoestrous (Chemineau et al., 2008; Palacios et al., 2023), confirming its efficacy in replicating natural cues for reproductive activation.

Among the nine recorded behaviours (Fig. 1), anogenital sniffing (AGS) was the most frequent, followed by approaching ewes (APE) and chasing ewes (CHE), with means values of 181.4, 132.1, and 101.9 observations per ram, respectively. Collectively, these accounted for 80.2 % of all observed activity, with L consistently exhibiting the highest values and C the lowest. Mount mating (MOM) was the least frequent behaviour (1.5 observations per ram), in agreement with Palacios et al., (2023), likely due to the absence of oestrus synchronisation and the seasonal reproductive quiescence of the ewes. Nevertheless, the introduction of males can trigger a follicular phase and LH surge without overt behavioural oestrus (Martin et al., 1986) suggesting that most mating opportunities may have occurred after the 14-day observation period.

Sexual activity (Fig. 2A) was significantly higher in the afternoon (47.7 %) than in the morning (26.7 %) or at night (25.6 %) ($\chi^2 = 119.6$; $p < 0.01$). This diurnal pattern agrees with previous findings that circadian rhythms mediated by melatonin reduce nocturnal sexual activity (Touitou et al., 2017; Wyse et al., 2018). AGS predominated at all times, particularly in the afternoon: 113 observations in L, 76.3 in C, 80.3 in L+C, and 101.5 in C+L. In L and C+L, APE occurred more frequently at night than in the afternoon, a pattern not observed in the remaining groups.

Daily activity (Fig. 2B) varied significantly ($\chi^2 = 471.6$; $p < 0.01$), with over 50 % of the 5707 observations occurring between days 2 and 5 post-stimulation, peaking on day 5 (15.2 %) and reaching the lowest level on day 12 (3.1 %). In L, AGS peaked on days 2, 4, and 5 (35.3, 44.0, and 42.7 observations, respectively), while APE peaked on days 4 and 5 (31.3 and 37.0). C showed low activity overall, with a modest AGS peak on day 11 (28.7). Mixed groups showed intermediate responses: L+C displayed AGS peaks on days 1, 4, and 5 (24.0, 24.0, and 23.3), and C+L peaked on days 0, 2, 3, and 4 (22.5, 33.0, 29.5, and 29.0).

3.2. Quantitative analysis of sexual activity

Mean frequencies for each behaviour are summarised in Table 1. Significant differences ($p < 0.05$) were observed for AGS, APE, urine sniffing (URS), and flehmen (FLE), with L showing the highest and C the lowest values. L+C ranked second in three of these variables, suggesting partial activation through social facilitation. AGS dominated across all groups, confirming its role as a key precursor to mounting and as a general indicator of sexual motivation (Roselli et al., 2004).

No significant differences emerged (Table 1) for CHE, pawing ewes (PAE), pushing ewes (PUE), mount attempts (MOA), or MOM, although PAE ($p = 0.060$) was more frequent in C. Total sexual activity differed significantly among groups ($p = 0.003$), with L showing the highest activity. This supports the view that photostimulated rams exhibit enhanced sexual behaviour when exposed to females during spring (Bronson, 1985; Maina and Katz, 1997). Social facilitation was evident in mixed groups, where both stimulated and non-stimulated rams maintained the highest level of sexual activity, likely mediated by neuroendocrine responses to social cues and influenced by dominance hierarchies (Bronson, 1985; Ungerfeld and González-Pensado, 2008).

Analysis by time of day (Table 2) confirmed the afternoon as the most active period, with L again maintaining the highest levels. Significant differences between groups were found in morning ($p = 0.036$) and night ($p = 0.026$) activity, with L being the most active and C+L the least active in the morning. Night activity decreased across all groups, although L maintained the highest frequency of behaviours.

Over time (Table 2), sexual activity peaked on day 0 for all groups except C, which peaked on day 8. Activity declined progressively thereafter, with the first week being the most active. This pattern indicates that the behavioural effects of the male stimulus are transient, diminishing without continued exposure to novel males or oestrous

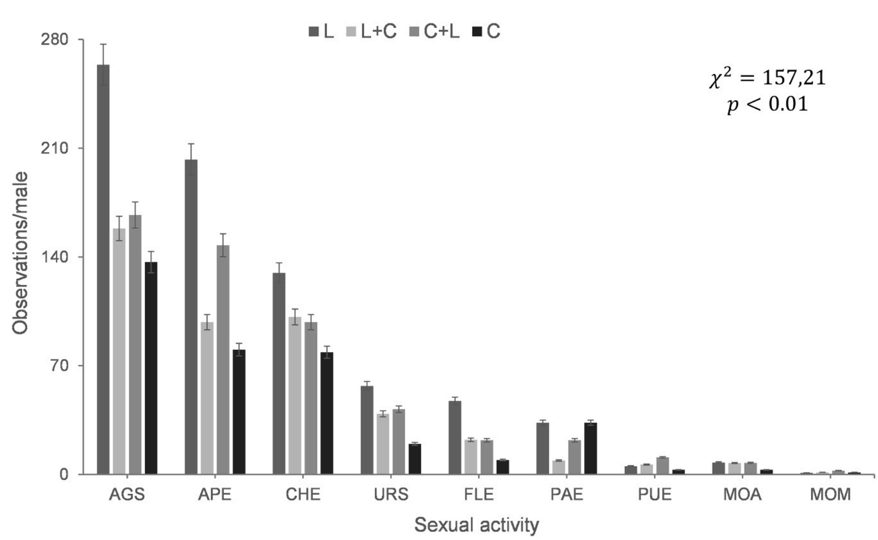

Fig. 1. Average number of sexual behaviour observations per ram across the four experimental groups; L: light-stimulated rams, C: control rams, L+C: light-stimulated rams exposed to control and C+L: control rams exposed to light-stimulated males. AGS: anogenital sniffing; APE: approaching ewe; CHE: chasing ewe; URS: urine sniffing; FLE: flehmen; PAE: pawing ewe; MOA: mount attempt; PUE: pushing ewe; MOM: mount mating.

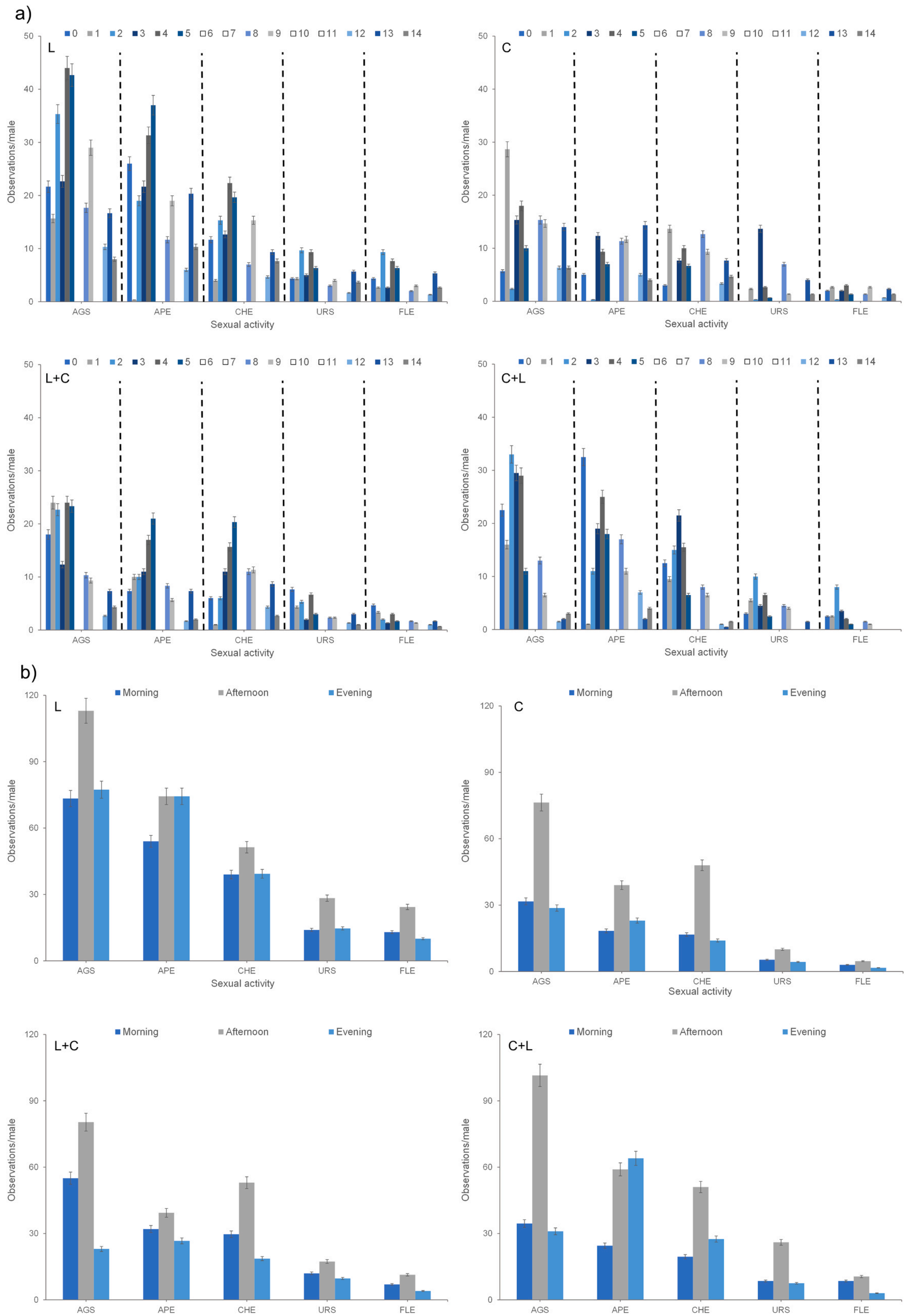

Fig. 2. A. Average number of observations per ram of selected sexual behaviours in the three time slots defined for each experimental group. B. Daily average observations of selected sexual behaviours per ram during the 14-day post-stimulation period, across the four experimental groups. AGS: anogenital sniffing; APE: approaching ewe; CHE: chasing ewe; URS: urine sniffing; FLE: flehmen.

Table 1
Mean number of sexual behaviours per ram and per 30-minute video segment across the four experimental groups.

Sexual activity	L	L+C	C+L	C	Total	Sig.
AGS	5.77 ± 0.588[a]	3.74 ± 0.490[ab]	3.59 ± 0.554[b]	3.50 ± 0.572[b]	4.24 ± 0.282	0.007
APE	4.44 ± 0.537[a]	2.31 ± 0.326[b]	3.17 ± 0.622[ab]	2.06 ± 0.366[b]	3.03 ± 0.238	< 0.001
CHE	2.84 ± 0.350	2.39 ± 0.317	2.11 ± 0.385	2.02 ± 0.346	2.37 ± 0.174	0.315
URS	1.25 ± 0.158[a]	0.92 ± 0.139[ab]	0.90 ± 0.179[ab]	0.50 ± 0.086[b]	0.91 ± 0.073	0.003
FLE	1.04 ± 0.126[a]	0.53 ± 0.092[b]	0.47 ± 0.137[b]	0.24 ± 0.052[b]	0.59 ± 0.055	< 0.001
PAE	0.73 ± 0.157	0.21 ± 0.061	0.47 ± 0.148	0.85 ± 0.296	0.57 ± 0.093	0.060
PUE	0.12 ± 0.051	0.15 ± 0.039	0.24 ± 0.150	0.08 ± 0.030	0.14 ± 0.035	0.502
MOA	0.17 ± 0.048	0.17 ± 0.101	0.16 ± 0.109	0.08 ± 0.028	0.15 ± 0.038	0.781
MOM	0.02 ± 0.013	0.03 ± 0.025	0.05 ± 0.035	0.03 ± 0.027	0.03 ± 0.012	0.848
Total	16.37 ± 1.690[a]	10.46 ± 1.209[b]	11.17 ± 1.682[ab]	9.37 ± 1.410[b]	12.04 ± 0.766	0.003

Data are mean ± SEM (standard error of the mean).
Within a row, means followed by the same letter are not significantly different ($P > 0.05$) according to Bonferroni's test.
AGS: anogenital sniffing, APE: approaching ewe, CHE: chasing ewe, URS: urine sniffing, FLE: flehmen, PAE: pawing ewe, PUE: pushing ewe, MOA: mount attempt, MOM: mount mating, Sig: significance.

Table 2
Mean number of sexual behaviours per ram and per 30-minute video segment across different times of day (morning, afternoon, night) for each experimental group and Temporal evolution of the mean number of sexual behaviours per ram and per 30-minute video segment throughout the 14-day observation period for each experimental group.

Sexual behaviours across different times of day						
Time slot	L	L+C	C+L	C	Total	Sig.
Morning	23.04 ± 4.18[a]	14.41 ± 2.78[a]	10.75 ± 3.03[ab]	11.25 ± 2.83[ab]	15.25 ± 1.71	0.036
Afternoon	23.85 ± 3.24[a]	17.05 ± 2.66[a]	19.54 ± 3.59[a]	16.56 ± 3.54[a]	19.30 ± 1.63	0.337
Night	9.73 ± 1.95[b]	4.38 ± 0.90[b]	6.16 ± 1.99[b]	4.04 ± 1.00[c]	6.27 ± 0.80	0.026
Sig.	< 0.01	< 0.01	0.002	< 0.01	< 0.01	
Sexual behaviours across different days post-stimulation						
Day post-stimulation	L	L+C	C+L	C	Total	Sig.
0	37.00 ± 6.78[a]	23.00 ± 5.38[a]	38.00 ± 13.57[a]	8.67 ± 5.03	25.64 ± 4.26	0.033
1	5.53 ± 2.81[b]	9.60 ± 3.91[ab]	8.90 ± 6.09[b]	10.07 ± 4.99	8.49 ± 2.14	0.869
2	19.00 ± 6.56[ab]	9.27 ± 3.13[ab]	13.79 ± 5.25[ab]	1.00 ± 0.52	11.42 ± 2.48	0.086
3	12.38 ± 3.88[ab]	6.76 ± 2.46[ab]	9.22 ± 2.80[b]	14.64 ± 7.05	10.32 ± 1.90	0.527
4	24.60 ± 3.84[ab]	14.50 ± 5.04[ab]	13.33 ± 4.06[ab]	10.31 ± 2.92	16.04 ± 2.12	0.075
5	20.11 ± 4.94[ab]	19.17 ± 4.67[ab]	9.75 ± 3.27[ab]	6.67 ± 3.01	15.00 ± 2.38	0.102
8	22.33 ± 11.69[ab]	18.67 ± 7.98[ab]	22.00 ± 15.10[ab]	24.50 ± 12.01	21.86 ± 5.34	0.986
9	18.83 ± 6.91[ab]	7.92 ± 2.91[ab]	7.38 ± 3.35[b]	10.25 ± 4.35	11.43 ± 2.47	0.320
12	8.44 ± 3.79[b]	3.78 ± 1.39[b]	3.33 ± 2.47[b]	5.44 ± 3.00	5.42 ± 1.42	0.595
13	9.94 ± 2.91[ab]	5.80 ± 2.10[ab]	2.67 ± 2.67[b]	8.25 ± 3.03	7.53 ± 1.45	0.468
14	15.57 ± 11.32[ab]	5.33 ± 3.17[ab]	6.00 ± 3.21[b]	9.33 ± 4.30	9.77 ± 3.81	0.770
Sig.	0.031	0.023	0.049	0.305	< 0.01	

Data are mean ± SEM (standard error of the mean).
Within a row, means followed by the same letter are not significantly different ($P > 0.05$) according to Bonferroni's test.
Sig.: Signification.

females.

4. Conclusions

Artificial photostimulation markedly enhanced sexual behaviour in rams during the first 14 days of the male effect, leading to higher levels of courtship activity compared with non-stimulated males. The observed behavioural convergence between photostimulated and non-stimulated rams when co-housed suggests that social facilitation plays a relevant role in sexual activation. Ewe anogenital sniffing emerged as the most consistent behaviour across all groups, indicating its potential as an early indicator of male sexual interest, whereas mounting was rare, likely reflecting the natural anoestrous of the ewes. Sexual activity followed a clear diurnal pattern, with minimal expression at night, which is consistent with circadian influences. Overall, these findings highlight the importance of integrating photoperiod manipulation and social context to optimise the male effect in reproductive management strategies for seasonal breeding systems.

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CRediT authorship contribution statement

Carlos Palacios: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pérez García Elena:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **José Alfonso Abecia:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Javier Plaza:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jaime Nieto:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carlos Palacios reports financial support, administrative support, equipment, drugs, or supplies, statistical analysis, and writing assistance were provided by University and Diputación of Salamanca. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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