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The isolation of females from males to promote a later male effect is unnecessary if the bucks used are sexually active

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Abstract

It has been suggested that female goats in permanent contact with males become refractory to their presence, and need to be previously separated from them for 40-45 days if the presence of bucks is to induce reproductive activity, ovulation and oestrous during seasonal anoestrous. The present study examines the reproductive response (ovulation and oestrus) and reproductive performance of does isolated from bucks for different periods before their reintroduction to male company. A total of 103 Payoya and Blanca Andaluza does were distributed into six treatment groups that required their isolation from males for different periods: 0 days (N=29), 5 days (N=15), 10 days (N=14), 20 days (N=16), 30 days (N=14) and 39 days (N=15). After this period they were introduced to sexually active bucks (ensured to be in this condition by keeping them under long days light treatment for three months), and oestrous activity was recorded daily by direct visual observation of the marks left by the marking harnesses worn by these males. Ovulation was confirmed via the plasma progesterone concentration (measured in blood samples taken twice per week). The ovulation rate was assessed by transrectal ultrasonography. Fecundity, fertility, prolificacy and productivity were also determined. The sexual behaviour of the males towards the females was also monitored on Days 0, 1, 2, 3, 4, 8 and 9 after their meeting with the latter. The length of the female isolation period had no effect on the percentage of does that responded to contact with the males, nor did it affect the oestrous response, fecundity, fertility or productivity. The males, however, undertook more ano-genital sniffing and nudging with the 5 day group females compared to those of the other groups ($P<0.05$). However, the sexual behaviour of the males changed as the days passed, with ano-genital sniffing becoming less common, and nudging, licking, sneezing and mounts with intromission more frequent on Days 8 and 9 than on Day 0, 1 and 2 after the sexes were reunited ($P<0.05$). These results show that the isolation of females is not necessary for an efficient male effect if the bucks used are sexually active. In addition, the sexual behaviour of the bucks changes as the time in contact with the does increases, but in general is not affected by the duration of female isolation.

Key words: goat, oestrous, ovulation, fertility, productivity, artificial photoperiod.

1. Introduction

The reproductive seasonality of goats living in subtropical and temperate latitudes is an important limitation to productivity. To counter this, reproduction needs to be induced during natural seasonal anoestrus [1, 2, 3, 4, 5]. The induction of the male effect via the re-introduction to males of does isolated from bucks has been shown an effective means of inducing female reproductive activity during this time of normal sexual rest [6, 7, 8, 9, 10, 11]. In fact, the sudden exposure of anovulatory does to bucks results in a rapid increase in their luteinising hormone (LH) pulse frequency, followed by a pre-ovulatory LH surge and ovulation [7, 8]. It has been suggested, however, that the duration of isolation from males, and the intensity of male sexual behaviour upon new contact, may influence the response of does [8].

Underwood et al. (1944) [12] were the first to propose that that anoestrous ewes in permanent contact with rams likely become refractory to male stimuli, and that they respond to rams only if conditioned by a period of total isolation from all male contact. Thereafter it was shown that a period of isolation from rams of 17-21 days was enough to induce ovulation upon the resumption of contact [13, 14]. However, just 24 h of isolation was sufficient to increase the LH pulsatility of ewes [15]. Shelton (1960) [16] then described the effect of introducing male goats to a group of does isolated from bucks at the end of seasonal anoestrus. Later, Chemineau (1987) [7] suggested that an isolation period of at least 3 weeks was necessary to induce ovulation in does re-exposed to bucks. Together, these findings suggest that, in both goats and sheep, females must be isolated from males if ovulation is to be induced via the male effect [8]. However, it has been suggested that such female isolation is not necessary when sexually active males are used [10, 17]. In addition, the ovulatory response of does to the male effect does not depend on male novelty [18]; in the latter work, a high proportion of does ovulated when exposed to either novel or familiar males - if both were sexually active.

The degree of sexual activity displayed by males in spring - when the male effect is employed - might influence the response of females to their presence. Bucks from Mediterranean and subtropical latitudes show a strong reduction in their plasma testosterone concentrations in spring, and consequently display only weak sexual behaviour from December to July (the months corresponding to the natural sexual rest period) [19, 20, 21]. Certainly, some authors report bucks employed during the sexual rest period to induce only a low percentage of does to ovulate [22, 23]. This limitation of the male effect can be circumvented by the use of bucks made sexually active by adequate photoperiod treatment. Experiments have shown that under such circumstances all previously isolated does exposed to photostimulated males ovulate, whereas <10% may do so when exposed to non-treated, sexually inactive males [23, 24]. Interestingly, in Mediterranean latitudes, three months of long days between the second fortnight of November until the second fortnight of February, followed by natural photoperiod conditions, also increases buck plasma testosterone concentrations and intensifies their sexual behaviour in March-April [20].

Given the capacity of adequately photostimulated bucks to induce ovulatory activity in does in seasonal anoestrus, it was hypothesized that prior female isolation may not be necessary for reproductive activities in such does to be stimulated. Further, the characteristics of this reproductive activity might not differ regardless of the duration of female isolation from males. These ideas were tested by monitoring the ovulatory/oestrous activities and reproductive performances of does exposed to males after different periods of separation.

2. Material and Methods

The study was conducted at the University of Huelva experimental farm (37º 20'N, 6º 54' W), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63).

2.1. Animals and management

The females used in this work (Payoya and Blanca Andaluza goats) were 3-4 year-old (adult) non-pregnant does (n=103). At the latitude where the work was performed, female anoestrus lasts from January-March to August-September [3, 5], while male sexual rest lasts from January-February to June-July [21, 25].

Over the experimental period, the does were maintained indoors and fed daily with lucerne hay, barley straw and commercial concentrate, according to INRA standards for maintaining adult weight and for providing adequate nutrition [26]. All animals had free access to water and mineral blocks containing trace elements and vitamins.

2.2. Preparation of females and males

Females

Figure 1 shows the experimental protocol. Initially the females were in contact with five adult vasectomised males. They were then distributed into six treatment groups that isolated them from males for different periods: 39 days (started February 19th; N=15; Group 39), 30 days (started February 28th; N=14; Group 30), 20 days (started March 10th; N=16; Group 20), 10 days (started March 20th; N=14; Group 10), 5 days (started March 25th; N=15; Group 5), and 0 days (in permanent contact with the vasectomised bucks; N=29; Group 0). These six groups were maintained in shaded open pens under natural day length during the entire experimental period. Both of the used breeds were distributed homogeneously in each group.

Males

Twelve entire males were exposed to 3 months of long days (16 h of light per day) from November 1st, and thereafter to natural photoperiodic conditions. These long days were provided via a mixture of natural light plus artificial light (at least 300 lux at the animals' eye level) from 6:00 to 8:00 h and from 19:00 to 22:00 h. This treatment stimulates testosterone secretion and sexual behaviour in bucks during March and April, i.e., the natural sexual rest period when control males are sexually inactive [20].

2.3. The male effect

On March 30th (Day 0), two males fitted with marking harnesses were placed in contact with each group of females to initiate the male effect and thus start breeding behaviour. The period of breeding lasted 38 days until May 7th.

2.4 Measurements

Detection of oestrous behaviour

During the period of breeding, oestrous activity was recorded by daily visual observation of the marks left by marking harnesses worn by the bucks [27].

Detection of ovulation

To monitor the ovarian cyclicity of the does before their introduction to the males (Day 0; March 30th), blood samples were collected once per week over three consecutive weeks and the plasma progesterone concentration determined. The does were deemed cyclic if their plasma progesterone concentration was >0.5 ng/mL in at least two consecutive samples. This has been shown indicative of ovulation [3, 28].

Ovulation was detected, and ovulation rates assessed, via the presence of corpora lutea observed during transrectal ultrasonography performed using an Aloka SSD-500 apparatus connected to a 7.5 MHz linear probe. This was conducted 6-8 days after the detection of oestrus [29]. The presence of corpora lutea was confirmed by the plasma progesterone concentration. Weekly blood samples were taken from the time of introduction to the males until the end of the study. Blood samples were collected by jugular venipuncture in tubes containing heparin. Plasma was obtained by centrifugation at 3500×g for 30 min and stored at -20°C until the hormone concentrations were measured. Plasma progesterone was determined in duplicate samples using a commercial enzyme linked immunoassay (ELISA) kit (Ridgeway Science Ltd., Gloucester, UK) in accordance with the manufacturer's instructions [30]. The mean intra-assay and inter-assay coefficients of variation were 6.6% and 9.9% respectively. The sensitivity of the assay was 0.1 ng/mL. Females with progesterone concentrations of ≥0.5 ng/mL were considered to have ovulated [27, 31].

Fecundity, fertility and productivity

Fecundity (nº pregnant does/nº does exposed to males) was determined via transrectal ultrasonography on day 45 after mating [32]. Fertility (percentage of goats kidding/does mounted by the males), prolificacy (number of kids born per female kidding) and productivity (nº kids born per female in each mating group) were also determined.

Sexual behaviour of bucks

One week before introducing the males to the females, the sexual behaviour of the former was assessed, recording nudging, ano-genital sniffing, mounting attempts, and the flehmen response over 5 min periods when the bucks were exposed to test females (i.e., not those in the treatment groups) in oestrus. All bucks displayed a similar sexual behaviour pattern; therefore, they were randomly allocated to serve the six groups of treated females.

After bringing the sexes into contact, the sexual behaviour of each buck (ano-genital sniffing, nudging, licking, sneezing, mounting attempts, and mounting with intromission) was observed for 30 min on Day 0 (i.e., the day of first contact), Day 1, Day 2, Day 3, Day 4, Day 8 and Day 9 post-introduction.

2.5. Statistical analysis

Results are reported as means ± standard error. The percentage of females that displayed oestrous behaviour, those that showed oestrous behaviour and ovulation, the fecundity and fertility values for each group, were compared using the Chi-squared test and Fisher's exact probability test. Ovulation rates and prolificacy were compared using the Mann-Whitney U test. Productivity, the date of ovulation, and the date of showing oestrous with ovulation, were compared using one-way ANOVA with the treatments as fixed effects. When differences between groups were observed, a Tukey test was performed. The sexual behaviour of the bucks during contact with the females of each group at different moments was compared using the independent two-sample t-test. Significance was set at $P<0.05$.

2.6. Ethical note

Trained personnel performed all procedures in strict accordance with Spanish guidelines for the protection of experimental animals (RD 53/2013), in agreement with European Union Directive 86/609.

3. Results

The percentage of goats in each group showing an ovarian response (mean for all groups 97%) and oestrous behaviour and ovulation (mean for all groups 88%) did not differ as a function of the time that the females spent isolated from males (Table 1). The mean interval between introduction to the male and the onset of oestrous behaviour and first ovulation was very synchronized (7.6 ± 0.7 days and 12.1 ± 0.7 days respectively) and did not differ significantly between groups (Table 1). Similar results were obtained for the interval between introduction to the males and mounting (9.9 ± 0.7 days; no significant difference between groups). Neither did it appear to have any effect on fecundity and fertility (70% for all groups), prolificacy (1.48 ± 0.06 kids born per female kidding) or productivity (0.92 ± 0.08 kids born per female in each group), with no significant differences between groups (Table 1).

Sexual behaviour of bucks

Few differences were seen between the groups in terms of male sexual behaviour (Fig. 2), although in Group 5 the number of nudging and licking events was higher ($P<0.05$). As the days passed since the sexes were reunited, the kind of behaviour shown by the males changed (Fig. 3). On Days 0, 1 and 2 ano-genital sniffing predominated, but thereafter the males' behaviour became enriched with more licking and sneezing events, more mounting attempts, and more mountings with intromission.

4. Discussion

The results of the present experiment show that, in goats, the reproductive activity and performance of does are unmodified by the time they spend isolated from males - if the bucks to which they are later introduced are sexually active. Indeed, the sudden presence of active males induced intense reproductive activity in the does; almost all the females (87%) in every isolation treatment group showed oestrous behaviour associated with ovulation. In addition, no differences were seen between these groups in terms of female fecundity, fertility, prolificacy or productivity.

These findings support the hypothesis that, in goats, the separation of females from males is not necessary for reproductive activities to be induced during seasonal anoestrus - as long as the bucks used are sexually active. This information is not only of use to managers of conventional goat production systems, but also to those managing organic systems in which no hormonal treatments are allowed (the present males were rendered sexually active by photoperiodic treatment alone).

Since the male effect was first reported in the 1940s [12], it has been assumed that females need to be isolated from male stimuli if the male effect is to successfully induce ovulation. It has been suggested that does become refractory to exposure to bucks, and thus need to be separated from them before trying to induce the male effect during the seasonal anoestrus. In the present experiment, nearly 100% of the females ovulated, most showed oestrous behaviour associated with ovulation, and the sexual response of the does was not modified by time they had spent isolated from males (0-39 days). These results agree with previous suggestions that, in goats, isolating females from males is unnecessary if sexually active males are used to induce the male effect [10, 17]. The experimental design used in the present study, however, reveals without ambiguity that the reproductive activity of does can be stimulated even when these have not been previously isolated from males.

Several studies report that photostimulated bucks are more efficient than untreated ones in stimulating sexual activity in anoestrus does [22, 24, 33]. In the present study, the photoperiod-treated bucks all showed active sexual behaviour. The vasectomised males in permanent contact with the females before the latters' isolation and later introduction to the active males, all displayed naturally low-level springtime sexual behaviour [25]. These sexually inactive bucks were unable to induce any reproductive activity in the does. It has recently been shown in both goats [34] and sheep [35] that using sexually active males can eliminate the effects of anoestrus. Together, these results highlight the importance of male sexual condition in inducing the male effect.

A criticism of the present experimental design may lie in the fact that the sexually active bucks to which the does were introduced would have been novel to them; this might have masked the effect of female isolation since they were not the same males present during the period prior to this separation. Our group recently demonstrated that the introduction of novel males to females already in contact with familiar males induces ovarian activity similar to that observed in the classical male effect [10]. Even, Muñoz et al. (2016) [18] recently demonstrated that photostimulated bucks induce sexual activity in seasonally anoestrous does independent of their familiarity with them. Consequently, the use of different males during the period prior to isolation and to induce the male effect should not have influenced the results.

No differences were seen between the different treatment groups in terms of fertility (50- 80%) or productivity (0.75 to 1.20 kids/doe). These results are similar to those reported by our group when using males 'activated' by inserting melatonin implants in the spring [10, 36]. They also show that such long day treatment of bucks not only activates sexual behaviour but improves sperm production and quality enough to achieve pregnancies in spring. In addition, Zarazaga et al. (2010) [20] reported that a photoperiod treatment similar to that used in the present work, activated male sexual behaviour in spring and improved sperm production and sperm quality compared to untreated bucks.

Male-female interactions were clearly not greatly modified by the time the does spent isolated from bucks. However, the behaviour displayed changed - as expected - as the days passed. After male introduction, most does experience a short ovarian activity and then enter oestrus. Thus, it would be expected that, during the early days after the sexes come into contact, the most common male behaviour would be ano-genital sniffing as the bucks try to detect females in oestrus. As time passed and the females came clearly into oestrus, this courtship behaviour became enriched, culminating in mating. These results agree with those described by Loya-Carrera et al. (2014) [37], who observed more mounting attempts and mounts with intromission on Day 8 following male introduction than on Days 0 or 1. Thus, in the present work, male-female interactions were not affected by the time the does spent isolated from males, probably because of the intense sexual activity of the bucks used.

5. Conclusions

Taken together, the present results show that bucks made sexually active by an artificial photoperiod can induce reproductive activity in does, independent of the time the latter are isolated from male company. Indeed, it would appear that the male effect can be induced even if does experience no previous isolation from males - as long as the inducing males are sexually active. Thus, if active males are used, goat livestock management can be simplified by obviating the need for female isolation. The fact that male sexual activity can be induced by photoperiod control means bucks thus treated could also be used to induce the male effect in organic goat-raising systems.

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Conflict of interest

None of the authors of this paper has any financial or personal relationship with any other person or organisation that might inappropriately influence or bias the content of the paper.

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Figure 1: Experimental design.

Figure 2. Sum of the types of male sexual advance experienced by females of each Group over the nine days of observation (observed over 30 min on each day). Males were rendered sexually active by exposure to long days (16 h of light by day) from November 15th to February 15th. Different letters on bars for the same variable indicate significant differences between Days at $P<0.05$.

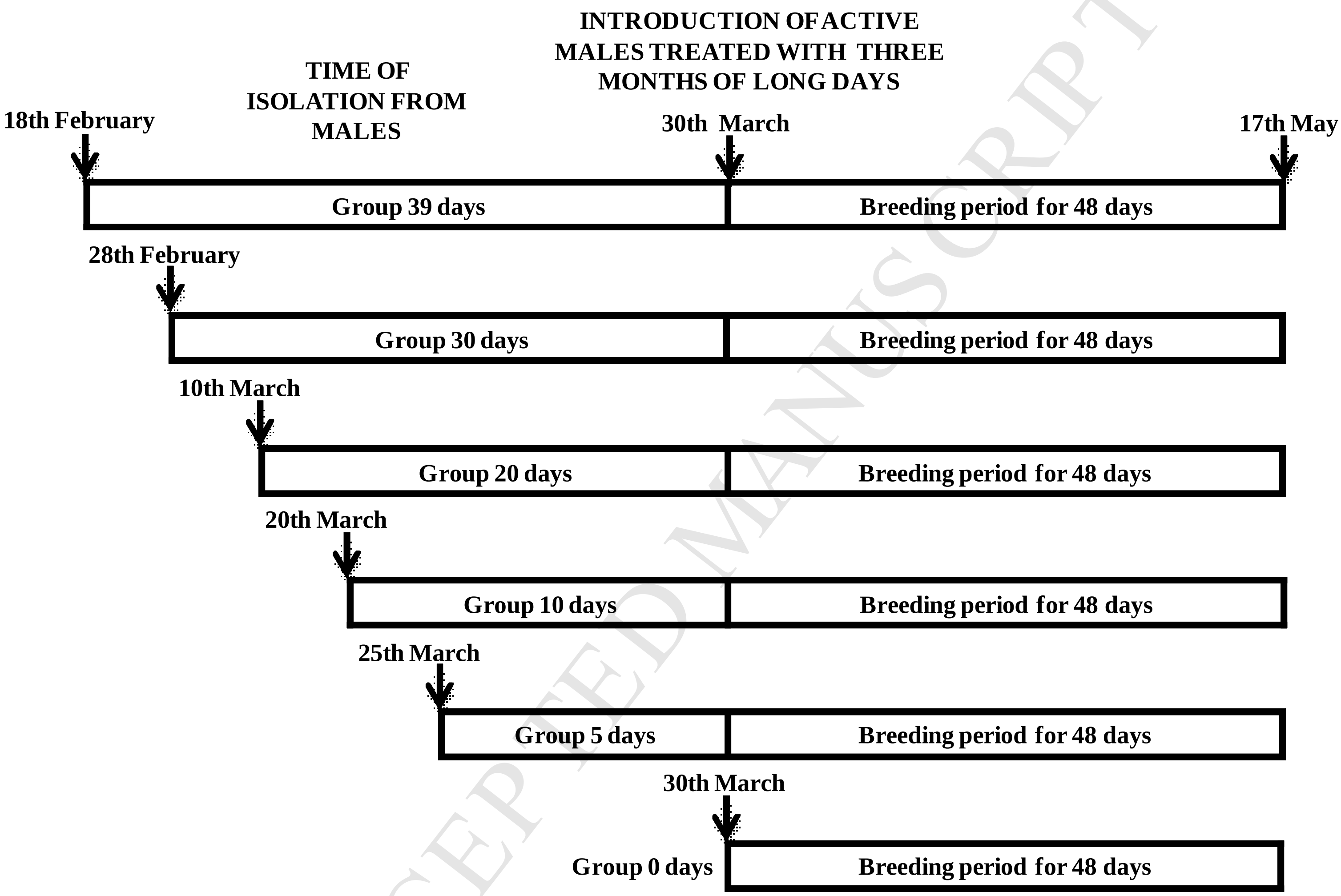
Figure 3. Types of male sexual advance experienced on Days 0 (day of introduction to males), 1, 2, 3, 4, 8 and 9 (observed over 30 min on each day) of Group 0, 5, 10, 20, 30 and 39 days. Males were rendered sexually active by exposure to long days (16 h of light by day) from November 15th to February 15th. Different letters on bars for the same variable indicate significant differences between Days at $P<0.05$.

Table 1: Reproductive results of females goats submitted to the male effect after an isolation period of: 39 days (Group 39), 30 days (Group 30), 20 days (Group 20), 10 days (Group 10), 5 days (Group 5), and 0 days (Group 0)

	39 days N=15	30 days N=14	20 days N=16	10 days N=14	5 days N=15	0 days N=29
Females showing ovulation (%)	100	100	100	100	100	90
Females showing oestrous and ovulation (%)	100	79	88	79	100	86
Interval male introduction 1st oestrous (days)	8.2 ± 1.3	6.9 ± 1.3	9.0 ± 3.2	7.3 ± 1.2	9.8 ± 2.2	6.3 ± 1.1
Interval male introduction 1st ovulation (days)	9.5 ± 1.5	10.2 ± 2.4	14.3 ± 3.2	15.1 ± 2.8	13.5 ± 2.7	11.4 ± 1.0
Interval male introduction and mounting (days)	9.5 ± 1.0	8.0 ± 1.0	10.2 ± 3.0	9.1 ± 0.1	11.7 ± 1.7	10.1 ± 1.5
Ovulation rate	1.29 ± 0.18	1.33 ± 0.10	1.83 ± 0.31	1.00 ± 0.00	1.50 ± 0.34	1.33 ± 0.10
Fecundity (%)	60	64	50	57	80	62
Fertility (%)	60	82	57	72	80	72
Prolificacy (kids born per female kidding	1.56 ± 0.18	1.56 ± 0.18	1.50 ± 0.19	1.38 ± 0.18	1.50 ± 0.15	1.44 ± 0.12
Productivity (kids born per female in the group)	0.93 ± 0.23	1.00 ± 0.23	0.75 ± 0.21	0.79 ± 0.21	1.20 ± 0.20	0.90 ± 0.15

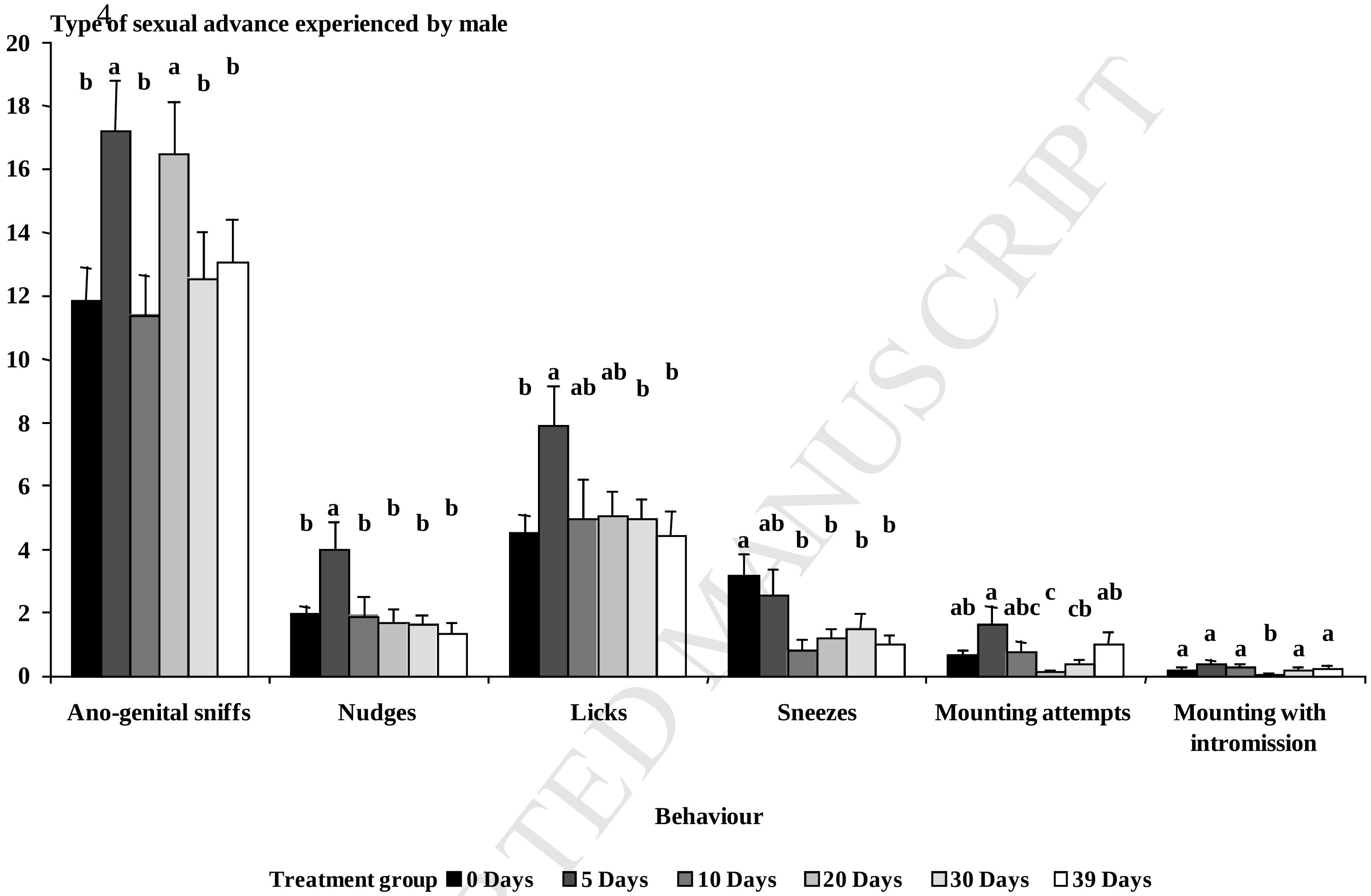
None of the reproductive variables studied were modified by the time of isolation ($P>0.05$).

1 Figure 1:



INTRODUCTION OF ACTIVE MALES TREATED WITH THREE MONTHS OF LONG DAYS
TIME OF ISOLATION FROM MALES
18th February
30th March
17th May
Group 39 days
Breeding period for 48 days
28th February
Group 30 days
Breeding period for 48 days
10th March
Group 20 days
Breeding period for 48 days
20th March
Group 10 days
Breeding period for 48 days
25th March
Group 5 days
Breeding period for 48 days
30th March
Group 0 days
Breeding period for 48 days

Figure 2.



Type of sexual advance experienced by male
20
18
16
14
12
10
8
6
4
2
0
b a b a b b
b a b b b b
b a ab ab b b
a ab b b b b
ab a abc c cb ab
a a a b a a
Ano-genital sniffs
Nudges
Licks
Sneezes
Mounting attempts
Mounting with intromission
Behaviour
Treatment group 0 Days 5 Days 10 Days 20 Days 30 Days 39 Days

Figure 3.

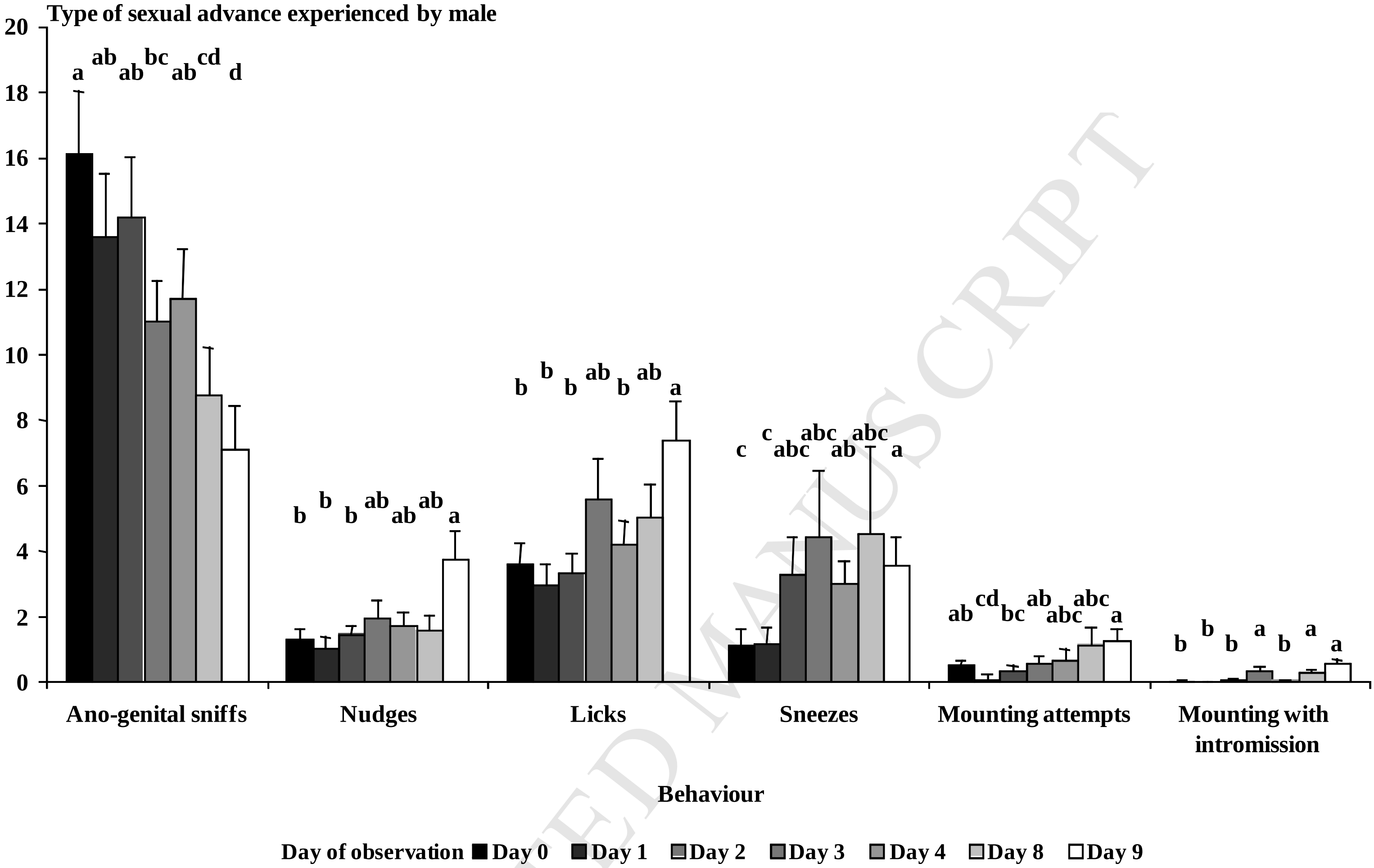
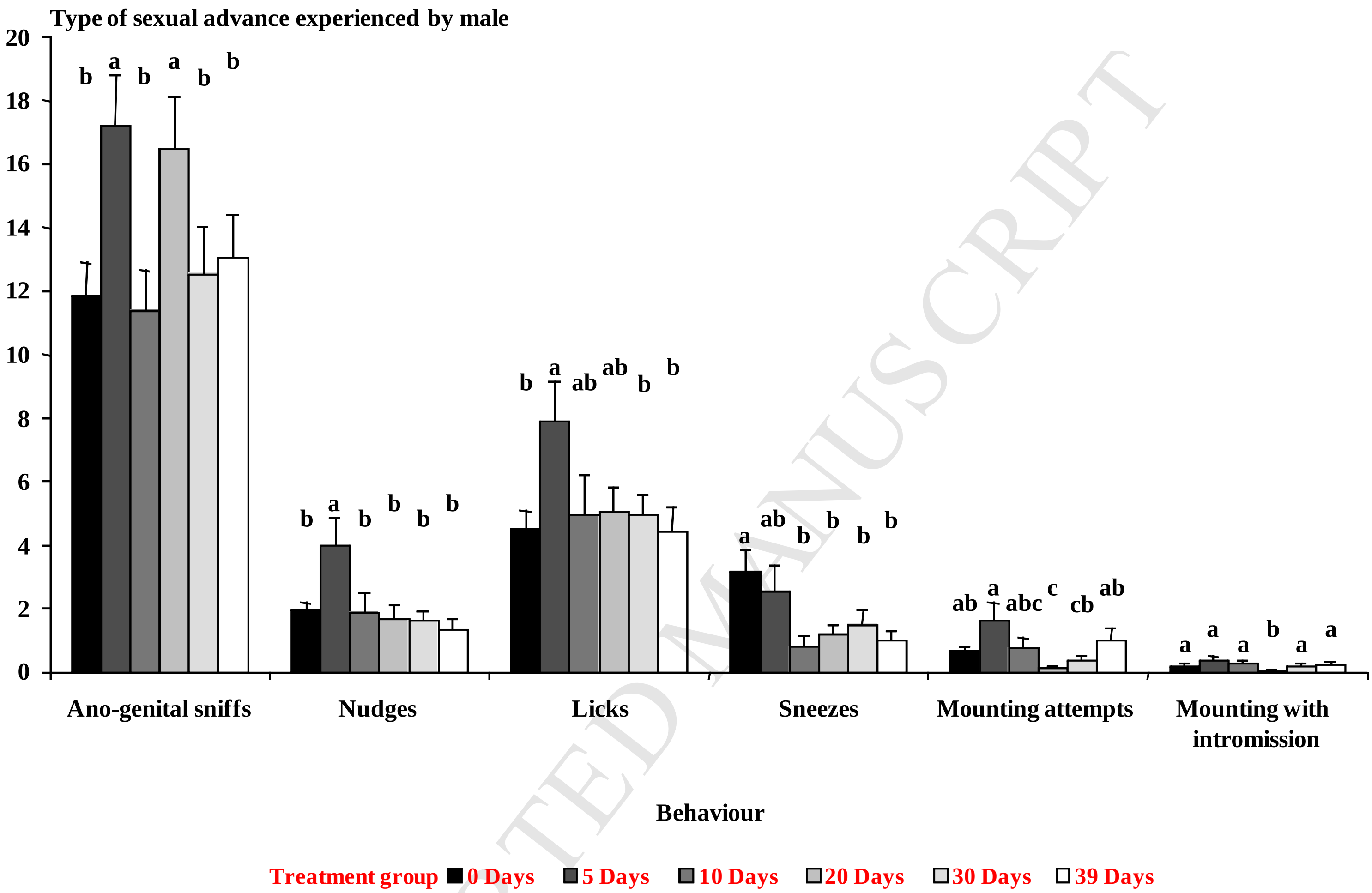

Type of sexual advance experienced by male
20
18
16
14
12
10
8
6
4
2
0
a ab ab bc ab cd d
b b b ab ab ab a
b b b ab b ab a
c c abc abc ab abc a
ab cd bc ab abc abc a
b b b a b a a
Ano-genital sniffs
Nudges
Licks
Sneezes
Mounting attempts
Mounting with intromission
Behaviour
Day of observation Day 0 Day 1 Day 2 Day 3 Day 4 Day 8 Day 9

Figure 2.


Type of sexual advance experienced by male
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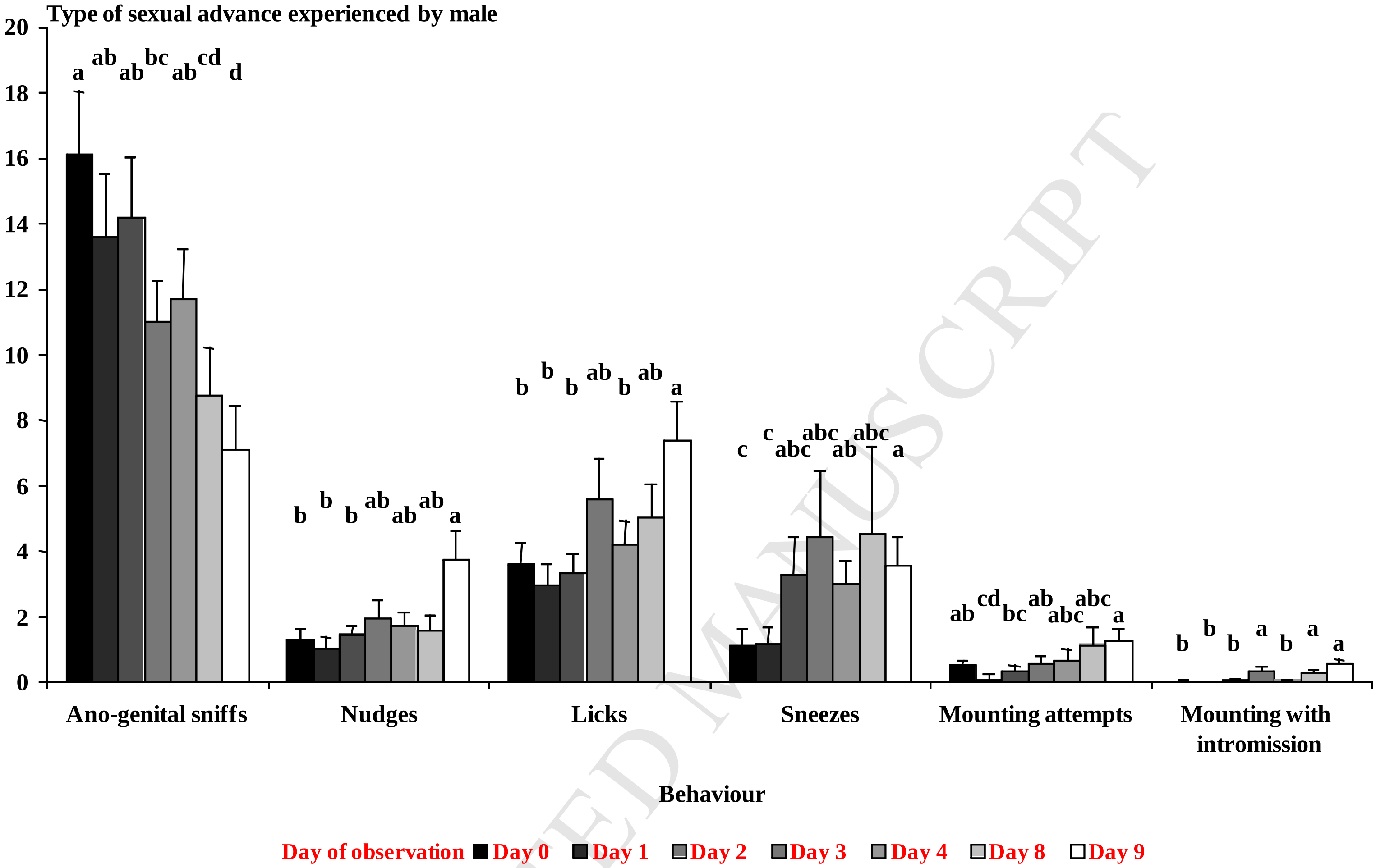
Sneezes
Mounting attempts
Mounting with intromission
Behaviour
Treatment group
0 Days
5 Days
10 Days
20 Days
30 Days
39 Days

Figure 3.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a ab ab bc ab cd d
b b b ab ab ab a
b b b ab b ab a
c c abc abc ab abc a
ab cd bc ab abc abc a
b b b a b a a
Ano-genital sniffs
Nudges
Licks
Sneezes
Mounting attempts
Mounting with intromission
Behaviour
Day of observation Day 0 Day 1 Day 2 Day 3 Day 4 Day 8 Day 9

Highlights

- Reproductive activity of bucks is reduced in spring when male effect is used.
- The male effect usually requires isolation between both sexes.
- It was hypothesized that prior isolation may not be necessary when we use active males.
- The length isolation period had no effect on none of the studied reproductive parameters.