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1 **Highly precocious activation of reproductive function in autumn-born goats (*Capra hircus*) by**
2 **exposure to sexually active bucks.**

3 Chasles Manon^a, Chesneau Didier^a, Moussu Chantal^a, Abecia José Alfonso^b, Delgadillo José
4 Alberto^c, Chemineau Philippe^a & Keller Matthieu^a

5 ^a*UMR Physiologie de la Reproduction et des Comportements, INRA, CNRS, IFCE, Université de*
6 *Tours, 37380 Nouzilly, France*

7 ^b*Instituto Universitario de Investigación en Ciencias Ambientales (IUCA), Departamento de*
8 *Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet, 177,*
9 *Zaragoza 50013, Spain*

10 ^c*Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro,*
11 *Torreón, Coahuila, Mexico*

12

13 Corresponding author: Matthieu Keller, Laboratoire de Physiologie de la Reproduction et des
14 Comportements, UMR CNRS, IFCE, INRA, Université de Tours, F-37380 Nouzilly, France.

15 Tel: +33 247 427 275 ; Fax: +33 247 427 743 ; Email: Matthieu.Keller@inra.fr

16

17 Mail adresses of authors: Manon Chasles, Chasles.Manon@gmail.com; Didier Chesneau,
18 Didier.Chesneau@inra.fr; Chantal Moussu, Chantal.Porte@inra.fr; José Alfonso Abecia,
19 alf@unizar.es; José Alberto Delgadillo, joaldesa@yahoo.com; Philippe Chemineau,
20 Philippe.Chemineau@inra.fr

21

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23 **Key words:** Puberty, Goats, Seasonal reproduction, Behavior, Estrous cycle, Ovulation

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28 **Abstract:**

29 Goats are seasonal breeders with the main cue controlling the timing of breeding season being
30 photoperiod. Hence, the season of birth impacts puberty onset: spring-born goats reach puberty in
31 autumn, at 7 months of age, while autumn-born goats reach puberty at 1 year during the next
32 reproductive season. The aim of this study was to determine whether exposure of autumn-born young
33 females to sexually-active males could counteract the delay in puberty onset observed in autumn-born
34 goats. Females exposed to sexually-active males (n=8) reached puberty earlier than isolated females
35 (n=8); with exposed females ovulating at a mean age of 3.5 months. To our knowledge, such
36 precocious puberty onset obtained through social stimulation has never been described in the
37 literature. Moreover, those exposed females exhibited estrus behavior for most ovulations. Our results
38 indicate that, in goats born out-of-season, exposure to sexually-active buck is really efficient to induce
39 an early puberty, suggesting that social interactions could have a crucial impact in the regulation of
40 pubertal transition.

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43 **Highlights:**

- 44 • Exposure to sexually-active bucks is a highly efficient way to induce ovulations in young does
- 45 • Puberty can be induced by exposure to male as early as 3.5 months in young female goats
- 46 • Exposure to sexually-active bucks failed to prevent a return to anestrus in young does

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54 1. Introduction

55 The pubertal transition is a key period which marks the beginning of the reproductive life.
56 During this period, the hypothalamic-pituitary-gonadal (HPG) axis that remained quiescent since early
57 post-natal life is reactivated and individuals acquire the physiological and behavioral ability for
58 reproduction. For females, puberty is usually defined as the occurrence of the first ovulation [1]. The
59 HPG axis is highly sensitive to internal and external factors [2], with one of the most important cues
60 being photoperiod.

61 Small ruminants are seasonal breeders; alpine goats kept under temperate latitudes exhibit a
62 succession of estrus and ovulatory cycles from early autumn to late winter [3]. Alpine bucks also
63 display seasonal variation of their sexual activity with a breeding period starting late summer and
64 finishing late winter [4]. For those seasonal breeders, photoperiod does not only affect adults'
65 reproductive functions but it also influences the start of the reproductive life of each individual by
66 regulating the onset of puberty. It has been shown that under both temperate [5] and subtropical [6]
67 latitudes, puberty will occur only during the breeding season. So, for females, the age at first ovulation
68 depends on their month of birth: spring-born goats reached puberty around 7 months of age during the
69 normal season of sexual activity, while does born out-of-season (autumn) reach puberty only around 1
70 year of age, due to the inhibitory effect of photoperiod on reproduction in spring and summer [5,6].
71 Therefore, out-of-season birth induces a significant delay of puberty of 5 months.

72
73 While photoperiod is considered to be the main environmental cue regulating reproductive
74 functions in seasonal breeders, other factors such as social interactions can also play an important role.
75 In small ruminants, the possibility to regulate reproduction by socio-sexual signals is well known,
76 especially the capacity of a male to induce ovulation when introduced into a group of anestrus
77 females [7,8]. This phenomenon, called the « male effect », has been well characterized in goats,
78 highlighting that the main limiting factor for an efficient male-effect is the level of sexual activity of
79 bucks [9]. The use of photo-stimulated bucks increases significantly the success of the male-effect.
80 The percentage of ovulatory females drops from 85-90% using photo-stimulated bucks to 5-10% using
81 non-stimulated bucks [10–13]. Hence, in goats, the reproductive axis of females seems to be highly
82 sensitive to signals provided by bucks.

83 Some studies reported that the presence of males could also affect the onset of puberty of young
84 females [ewes: 14–16; goats: 17,18]. This highlights that social factors can affect the immature
85 gonadotropic axis by inducing its precocious reactivation. In goats, the level of sexual activity of
86 males is crucial to induce precocious puberty in young females, as exposure to castrated bucks fails to
87 impact the age at puberty [18]. Nevertheless, most of the studies investigating pubertal induction in
88 ruminants were conducted with spring-born young females, so little information is available about the
89 possibility of modifying the onset of puberty in autumn-born females which are known to have a
90 delayed puberty. Recently, one study conducted in sheep highlighted that exposure to stimulated rams

91 was efficient to induce a precocious puberty in autumn-born ewes [19]. Females exposed to sexually-
92 active rams were pubescent 1.4 months before the control group. In our study, we tested the
93 hypothesis that exposure to sexually-active bucks will induce the onset of puberty of autumn-born
94 females earlier than in females isolated from bucks. In a previous study carried out in spring-born
95 females we highlighted that exposure to inactive bucks had no impact on the female pubertal transition
96 [18]. Hence, in this experiment, we choose to not include a group of female exposed to inactive bucks.

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98

99 2. Material and Methods

100

101 2.1 Animals

102 Experiments were carried out from September 2016 to April 2017 in Nouzilly, France (latitude
103 47° 32N and longitude 0° 46E) on alpine goats (*Capra hircus*). We used a total of 26 animals for this
104 study, 16 juvenile females and 10 sexually experienced adult males. Young females were born between
105 August 24th and September 4th. They were weaned at 2.5 month old and one week later they were
106 divided into two groups balanced for body weight. Sisters were separated to avoid any genetic effect.
107 Animals were fed daily with barley straw, lucerne hay and commercial concentrate, with free access to
108 water and mineral blocks. All procedures were performed in accordance with the European directive
109 2010/63/EU on the protection of animals used for scientific purposes and approved by an ethical
110 committee for animal experimentation (CEEA VdL, Tours, France, n°2016091910327211).

111

112 2.2 Photoperiodic treatment

113 To ensure that females were permanently exposed to sexually active bucks (SAB), the males
114 were divided into three groups and two of them were successively submitted to a photoperiodic
115 treatment of long days. This treatment consists of an exposure to artificial long days (16 hours of light
116 and 8 hours of darkness per day) for 80 days in a light proof building. Light intensity in the lightproof
117 building was at least 300 lx at the level of the eyes of the animals. Following this light treatment,
118 bucks were moved into open barns and exposed to the natural photoperiodic conditions.

119 One group of bucks was exposed to artificial long days from September 15th to December 1st
120 (SAB2, n=3) and another from November 4th to January 23rd (SAB3, n=3). Six weeks after, they were
121 exposed to females: from January 15th to March 5th for SAB2 and from March 6th to April 24th for
122 SAB3. SAB1 (n=4) bucks were used to stimulate females during the breeding season (from November
123 22nd to January 15th), hence they were naturally sexually actives and did not need to be stimulated
124 with a photoperiodic treatment. This photoperiodic treatment is known to be efficient in inducing a
125 sexually-active state in males during the non-breeding season after 45 to 60 days following the switch

126 of exposure from artificial long days to natural photoperiod [4,10]. The level of sexual behavior of
127 photo-stimulated bucks has been shown to be as intense as the one of naturally sexually active bucks
128 [20].

129 To evaluate the efficiency of the photoperiodic treatment, testicular volumes were assessed at
130 the end of the photoperiodic treatment and during the first week of exposure to females. Measures
131 were taken by the same operator to avoid inter-individual variations [21,22].

132

133 2.3 Pre-pubertal exposure to bucks

134 One group of 8 females was exposed to bucks (STIM) just after weaning while the other group
135 of 8 females remained isolated (ISOL) from any male. In the STIM group, exposure to bucks was
136 continuous behind a fence from November 22nd until April 24th. Throughout this period, we allowed
137 direct contact between bucks and does for 1 hour, 3 times a week; in this case, males were fitted with
138 an apron to prevent intromissions and conceptions. During those close interactions, we observed the
139 behavior of both males and females to detect estrus behavior and to ensure the sexually-active state of
140 the bucks. Ano-genital sniffing, flehmen and lateral approaches, indicative of buck behavior, were
141 measured. The number of contacts between male and females was quantified to ensure that the bucks'
142 and does' motivation to interact were similar throughout the experiment. A doe was considered in
143 estrus when it showed a state of excitement during which it vocalized more, wagged her tail and
144 accepted the mount by the buck.

145

146 2.4 Evaluation of puberty onset and weight gain

147 Age at puberty was assessed by the determination of the onset of ovulatory activity.
148 Ovulations were detected by measuring plasma progesterone (P4) concentrations twice a week. Blood
149 samples were obtained by jugular venipuncture from all females from the age of 3.5 months till the
150 age of 7.5 months in 5mL tubes containing heparin. Plasma was obtained after 30 minutes of
151 centrifugation at 3500g and concentration of plasma progesterone was determined in samples using an
152 immunoenzymatic assay as described previously [23]. Sensitivity of this assay was 0.25ng/mL. The
153 mean intra-assay and inter-assay coefficients of variation were <10%. Females with progesterone
154 concentrations ≥ 0.5 ng/mL in 2 consecutive samples were considered to have ovulated and then to be
155 pubescent [24,25]. Throughout the experiment, females were also weighed monthly.

156

157 2.5 Statistical Analysis

158 Results are reported as mean \pm SEM (standard error of the mean). A Shapiro-Wilk test has
159 been performed to test the data distribution. If data were normally distributed ($p > 0.05$) a parametric

160 test was performed, otherwise we used non-parametric tests. Weight data were compared between
161 groups using a two-way repeated-measures ANOVA. Intra-group variations of the testicular volume
162 were performed using paired Student t test and inter-groups variations were analyzed by the Kruskal-
163 Wallis test. The age at puberty was examined using a Kaplan-Meier survival analysis and differences
164 between groups were compared by the log rank test. Behavior of bucks was analyzed using the
165 Kruskal-Wallis test followed by multiple comparisons using the Tukey-Type test (« nparcomp »
166 package). **Number of cycles was** analyzed using the Mann-Whitney test and percentages of cycling
167 goats were compared using Fisher exact tests. Area under the curve (AUC) of progesterone secretion
168 was calculated for each group following the trapezoidal rule, comparison between groups was
169 performed by a Mann-Whitney test. All **analyses** were performed using R software [26]. Statistical
170 significance was set at $p < 0.05$.

171

172

173 3. Results and discussion

174

175 The aim of this study was to investigate the consequences of exposure to sexually active bucks
176 on the onset of puberty of female goats born out-of-season. We observed that goats exposed to
177 sexually active bucks had an earlier first ovulation than isolated goats ($p < 0.01$, **Fig. 1A**). On
178 December 19th, 5/8 of STIM females were considered pubescent while none of the ISOL females
179 reached puberty; at the end of the experiment (late April), all STIM females were pubescent but only
180 half of the ISOL females. In our study, young females exposed to sexually active bucks were
181 pubescent at a mean age of 112 days (3.6 months).

182 Our results are consistent with the only other study in ruminants in which induction of
183 precocious puberty in autumn-born females by sexually-active males occurred in sheep, with an
184 advance of 1.4 months. In this case, teased females were pubescent at a mean age of 7.8 months while
185 controls females reached puberty at 9.2 months [19]. This difference **from our findings** can be partly
186 explained by the different experimental design; indeed, we chose to expose females to bucks at
187 weaning (3 months). This was earlier than in this previous study in which rams were introduced when
188 females were 5.5 month old. In our experiment, more than 85% of goats exposed to sexually active
189 bucks reached puberty within a month after the first contact with males (**Fig. 1A**). We **might predict**
190 **that if ewes had been exposed to rams earlier, they might** have reached an even earlier puberty.
191 Interestingly we observed a great synchronicity of the first ovulation in the group exposed to sexually-
192 active males: all STIM females reached puberty in a range of 3 weeks, while in the control group only
193 50% reached puberty over the 5 months of the experiment (**Fig. 1A**). We have already observed this
194 **three-week** delay between the beginning of exposure to sexually-active **males** and the ovulatory
195 response of young females in our previous study conducted with young does born in season [18].

196 The difference in the age at puberty that we observed here between STIM and ISOL groups is

197 not due to other factors such as the metabolic state, which can impact the age at puberty. Indeed,
198 regarding the body weight variation of females, a strong effect of time ($F_{(7,98)}= 299.9$, $p<0.001$) and a
199 treatment-by-time interaction were detected ($F_{(7,98)}= 3.4$, $p<0.01$) but no group effect ($F_{(1,14)}= 0.58$,
200 $p=0.46$) can be observed (**Supplemental Fig. 1**). One single difference was observed between groups
201 for the month of April, STIM females were heavier than ISOL females ($39.88 \pm 2.5\text{Kg}$ versus $34.75 \pm$
202 2.5Kg ; $p<0.05$). Hence, no difference in body weight was detected between groups when they **started**
203 **ovulating, thus** this factor is therefore not responsible for the precocious puberty observed in females
204 exposed to sexually active male.

205
206 In this study, **we not only assessed the age at first ovulation, but we also characterized the**
207 **subsequent estrous cycle expression** of females. All young goats exposed to sexually-active bucks
208 **remained** cyclic after the first ovulation, with at least two other cycles similar to those observed in
209 mature goats [27]. In the ISOL group, only two females exhibited 2 cycles or more. Furthermore, for
210 STIM females, 28 ovulations were detected by progesterone levels, in most **cases** (20/28), females
211 **exhibited estrus** behavior few days before (**Fig. 1B**). In young goats, we observed, **as was expected**,
212 that most first ovulations are silent; i.e. that there is no **estrus** behavior associated with ovulation [28].
213 Observation of **estrus behavior is still a commonly used approach** to determine if a female is
214 pubescent. According to our results, this method would fail to detect pubescent females, females that
215 only had one cycle and that did not show any sign of **estrus** behavior which is the case of 3 out of 8 **for**
216 our isolated females.

217 **Because** STIM females had more ovulations than ISOL females, the area under the curve (AUC)
218 calculated for progesterone concentrations along the experiment (**Fig. 1C**) was significantly higher in
219 the STIM group than in the ISOL group ($p<0.01$). This difference is not only due to the **advancement**
220 **of first ovulation, but also because** exposure to males also induces the maintenance of the ovulatory
221 cyclicity. Indeed, females continue cycling 3 weeks later when exposed to sexually-active bucks than
222 when isolated from bucks (**Fig. 1D**). The period within which all females from a group were cycling
223 was 8.5 weeks in the STIM group and was only 1 week in the ISOL group. The percentage of cycling
224 goats was significantly higher in the STIM group for late-December and mid-February (**Fig. 1D**,
225 $p<0.05$). However, this enduring cyclicity ended late March. Our first hypothesis was that this was due
226 to the bucks no longer being sufficiently sexually active to stimulate females. However, the analysis of
227 the testicular volume of bucks used at this time of the experiment (SAB3) does not show any
228 difference when compared to the testicular volume of bucks from the two other groups while exposed
229 to females (**Fig. 2**; $p>0.05$). The photoperiodic treatment induced an increase of 18% of the testicular
230 volumes in both treated groups (SAB2 and SAB3). Comparing the behavior of bucks among the three
231 groups, no significant differences in the number of physical contacts between males and females were
232 detected (**Fig. 3**). An increase of the occurrence of appetitive behaviors is observed in SAB3 bucks,
233 hence those bucks exhibit more ano-genital sniffing than the 2 other groups (SAB1-SAB3: $p<0.05$;

234 SAB2-SAB3: $p < 0.01$) and more flehmen than SAB1 ($p < 0.05$). No significant difference was detected
235 in the occurrence of lateral approaches among bucks' groups (**Fig. 3**). Therefore, we conclude that, for
236 STIM females, the termination of estrous cycles was due to an inhibitory effect of photoperiod rather
237 than a lack of stimulation. Indeed, in goats, the anestrus season starts in March when day length
238 increases [29], providing an inhibitory signal to the reproductive axis.

239
240 Those results are however not consistent with a recent study [30] that raised the possibility of
241 continued estrous cycle expression due to continuous exposure to sexually active bucks. However, in
242 our experiment, while exposure to active bucks caused expression of estrous cycles, it failed to totally
243 override the anestrus period. The difference between the two studies can be explained by several
244 factors. First, magnitude of photoperiodic variations was different. The study by Delgadillo et al [30]
245 was conducted in Northern Mexico, where day length varies from 10 h at the winter solstice to 14 h at
246 the summer solstice. Under temperate latitudes variations are more important with, at the same dates, a
247 photoperiod varying from 8 h to 16 h. The greater photoperiodic variation observed under temperate
248 latitudes may result in a deeper anestrus and a stronger inhibitory effect of photoperiodic stimuli.
249 Social factors could then be overridden by photoperiodic input. Another difference between the two
250 studies was the level of sexual experience of females. The study of Delgadillo et al [30] used only
251 sexually experienced multiparous adult goats. In our study, females were younger and had a very brief
252 experience with the male (i.e. continuous exposure behind a fence for 4 months with only 3 hours per
253 week of direct contact and no copulation). It is known that sexual experience can impact the quality of
254 the female's response to the signal of the male. For example, multiparous ewes have a better response
255 to a male-effect than nulliparous ewes [31]. In goats, results obtained during a male-effect experiment
256 show in some cases a better response with multiparous females: 92% of those females exhibit estrus
257 behavior while only 16% of inexperienced females do so [32].

258
259 To our knowledge, we report for the first time that puberty of autumn-born young goats can be
260 significantly advanced by exposure to sexually-active bucks, without any hormonal treatment. The
261 presence of active bucks since weaning can induce an acceleration of the reactivation of the female
262 reproductive axis enabling young goats to ovulate, maintain a normal cyclicity (i.e. regular cycles of
263 approximately 21 days) and express an adequate estrus behavior as early as 3.5 months of age. This
264 result is highly original as the commonly accepted age at puberty for goats born in spring is 7 months
265 and one year for goats born in autumn. It highlights the fact that the gonadotropic axis is highly
266 sensitive to male sensory cues even at a young age. The presence of bucks also helped maintain the
267 cyclicity in young females but failed to prevent the anestrus season, as has been observed in mature
268 females.

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276

277 **Competing interests:**

278 No competing interests declared.

279

280 **Authors contribution:**

281 M. Chasles and M. Keller designed the experiments, performed the results analysis and wrote the
282 manuscript. P. Chemineau, J-A. Abecia and J-A. Delgadillo corrected the manuscript. C. Moussu and
283 D. Chesneau helped during animal experimentations and corrected the manuscript.

284

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386

387 **Figure legends**

388

389 **Figure 1. Physiological response of young goats exposed (STIM) or not (ISOL) to sexually**
390 **active bucks.** Blood samples for progesterone were collected twice a week from mid-December to
391 late-April. (A) Cumulative proportions of young goats reaching puberty from ISOL (n=8) and STIM
392 (n=8) groups. Females were considered pubescent when progesterone levels $\geq 0.5\text{ng/mL}$ in two
393 consecutive samples. Data were analyzed using log-rank test (**p < 0.001). (B) Representative
394 patterns of the cyclicity of females. STIM females were submitted to direct contact with bucks 3 hours
395 a week, estrus behavior (indicated by arrows) was determined during those close interactions. (C)
396 Comparison of the cumulative serum progesterone secretion expressed by area under the curve (AUC,
397 arbitrary unit). Stars indicate significant differences between groups using a Fisher exact test (**p <
398 0.01). (D) Percentage of pubescent females cycling in the STIM (n=8) and ISOL (n=4) groups. Stars
399 indicate significant differences between groups using a Fisher exact test (*p < 0.05).

400

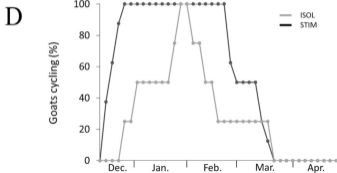
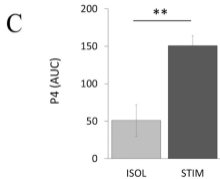
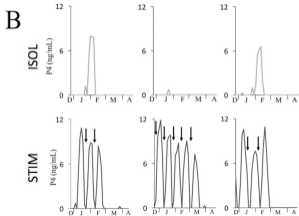
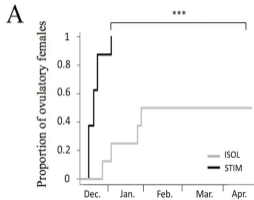
401 **Figure 2. Variation of the testicular volume.** Measure of the testicular volume was assessed by
402 orchidometry the first day of exposure to females for each group of bucks: SAB1 (n=4), SAB2 (n=3)
403 and SAB3 (n=3). For SAB2 and SAB3 bucks, a measure was also performed the last day of the long
404 day treatment. Results are presented as mean \pm SEM. Stars indicate significant differences within a
405 group using the paired Student t test (*p < 0.05). Different letters denote a significant difference
406 between groups using a Kruskal-Wallis.

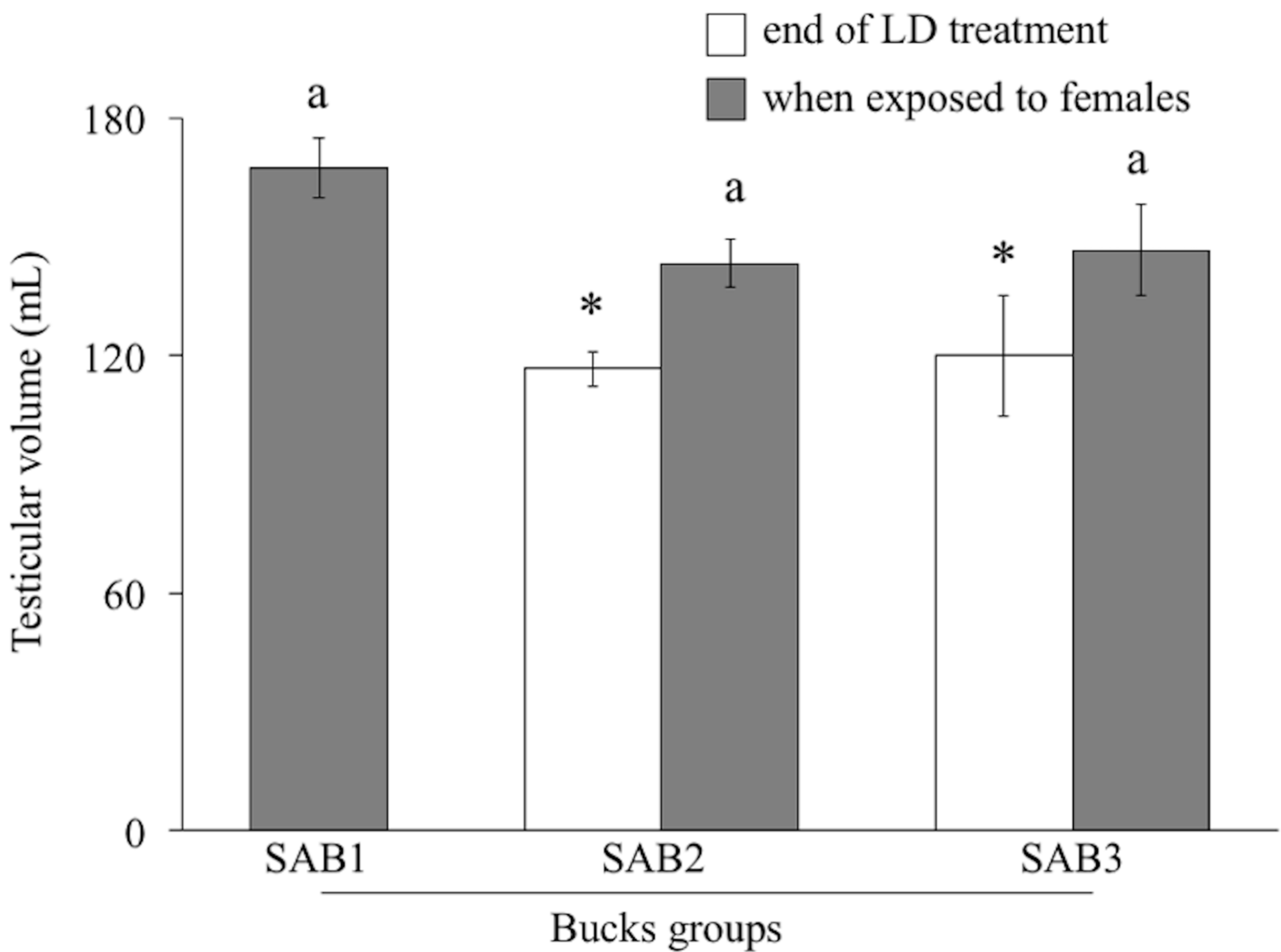
407

408 **Figure 3. Occurrence of socio-sexual behaviors of the three groups of bucks.** Behavior was
409 observed for 1h three times a week when a single buck was introduced into the females' pen. SAB1
410 (n=4) were used from November 22nd to mid-January, SAB2 (n=3) from mid-January to mid-March
411 and SAB3 from mid-March to late-April. Results are presented as the mean \pm SEM occurrence of each
412 behavior per hour. Stars indicate significant differences between groups using a Kruskal-Wallis test
413 (*p < 0.05, **p < 0.01).

414

415 **Supplemental Figure 1. Changes in body weight in female kids from birth to 7 months of**
416 **age.** Females from the 2 groups, ISOL (grey line; n=8) and STIM (black line; n=8) were weighed
417 monthly. Data are presented as mean \pm SEM. Stars indicate significant differences between groups
418 using a two-way repeated-measures ANOVA followed by pairwise t-test (*p < 0.05).





Occurrence of sexual behaviors
(mean \pm SEM)

