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

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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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## 1. Introduction

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

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

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

Some studies reported that the presence of males could also affect the onset of puberty of young females [ewes: 14–16; goats: 17,18]. This highlights that social factors can affect the immature gonadotropic axis by inducing its precocious reactivation. In goats, the level of sexual activity of males is crucial to induce precocious puberty in young females, as exposure to castrated bucks fails to impact the age at puberty [18]. Nevertheless, most of the studies investigating pubertal induction in ruminants were conducted with spring-born young females, so little information is available about the possibility of modifying the onset of puberty in autumn-born females which are known to have a 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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## 99 2. Material and Methods

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### 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 24<sup>th</sup> and September 4<sup>th</sup>. 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).

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### 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 15<sup>th</sup> to December 1<sup>st</sup>  
120 (SAB2, n=3) and another from November 4<sup>th</sup> to January 23<sup>rd</sup> (SAB3, n=3). Six weeks after, they were  
121 exposed to females: from January 15<sup>th</sup> to March 5<sup>th</sup> for SAB2 and from March 6<sup>th</sup> to April 24<sup>th</sup> for  
122 SAB3. SAB1 (n=4) bucks were used to stimulate females during the breeding season (from November  
123 22<sup>nd</sup> to January 15<sup>th</sup>), 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 22<sup>nd</sup> until April 24<sup>th</sup>. 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  $\geq 0.5$ 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  $p < 0.05$ .

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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 19<sup>th</sup>, 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  $2.5\text{Kg}$ ;  $p<0.05$ ). 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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**Competing interests:**

No competing interests declared.

**Authors contribution:**

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

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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  $\geq 0.5$ 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 22<sup>nd</sup> 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).







