Received: 22 November 2022 | Revised: 9 March 2023 | Accepted: 17 April 2023

DOI: 10.1111/jne.13284

ORIGINAL ARTICLE

Journal of Neuroendocrinology WILEY

Male-induced early puberty correlates with the maturation of arcuate nucleus kisspeptin neurons in does

Maxime A. Meunier[1] | Chantal Porte[1] | Kévin Poissenot[1] | Hélène Vacher[1] | Morgane Brachet[1] | Pablo Chamero[1] | Massimiliano Beltramo[1] | José A. Abecia[2] | José A. Delgadillo[3] | Philippe Chemineau[1] | Matthieu Keller[1]

[1]UMR Physiologie de la Reproduction et des Comportements, INRAE, CNRS, IFCE, Université de Tours, Nouzilly, France

[2]Departamento de Producción Animal y Ciencia de los Alimentos, IUCA, Universidad de Zaragoza, Zaragoza, Spain

[3]Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Torreón, Mexico

Correspondence
Matthieu Keller, UMR Physiologie de la Reproduction et des Comportements, INRAE, CNRS, IFCE, Université de Tours, 37380 Nouzilly, France.
Email: matthieu.keller@inrae.fr

Funding information
Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement; Région Centre Val de Loire

Abstract

In goats, early exposure of spring-born females to sexually active bucks induces an early puberty onset assessed by the first ovulation. This effect is found when females are continuously exposed well before the male breeding season starting in September. The first aim of this study was to evaluate whether a shortened exposure of females to males could also lead to early puberty. We assessed the onset of puberty in Alpine does isolated from bucks (ISOL), exposed to wethers (CAS), exposed to intact bucks from the end of June (INT1), or mid-August (INT2). Intact bucks became sexually active in mid-September. At the beginning of October, 100% of INT1 and 90% of INT2 exposed does ovulated, in contrast to the ISOL (0%) and CAS (20%) groups. This demonstrated that contact with males that become sexually active is the main factor prompting precocious puberty in females. Furthermore, a reduced male exposure during a short window before the breeding season is sufficient to induce this phenomenon. The second aim was to investigate the neuroendocrine changes induced by male exposure. We found a significant increase in kisspeptin immunoreactivity (fiber density and number of cell bodies) in the caudal part of the arcuate nucleus of INT1 and INT2 exposed females. Thus, our results suggest that sensory stimuli from sexually active bucks (e.g., chemosignals) may trigger an early maturation of the ARC kisspeptin neuronal network leading to gonadotropin-releasing hormone secretion and first ovulation.

KEYWORDS
goats, kisspeptin, ovulation, puberty, sociosexual relationships

1 | INTRODUCTION

Puberty is a complex process, marking the attainment of fertility and the onset of reproductive life. This transition corresponds to the reactivation of the hypothalamic–pituitary-gonadal (HPG) axis leading to gonadotropin-releasing hormone (GnRH) secretion.[1,2] Small ruminants, such as sheep and goats, are short-day breeders. The female breeding season in goats begins in early autumn and lasts until late winter.[3] In these species, many internal and external factors can influence the onset of puberty.[4] Thus far, photoperiod was considered the major environmental factor prompting the onset of puberty. Indeed, in seasonal breeds of goats, fertility is naturally achieved during the first breeding season.[5]

However, the importance of sociosexual interactions has been recently reconsidered as part of the control of reproductive function in small ruminants.[6] In the last century, male-induced ovulation was described in adult anoestrus sheep[7] and goats.[8] In this phenomenon, called the "male effect",[9] male sexual activity was the main factor triggering the reactivation of the female gonadotrope axis. Indeed, exposure to sexually inactive males failed to induce œstrus and ovulation in adult seasonally anoestrus females.[10] However, the role of male sexual activity in other situations, such as female pubertal transition, has been less characterized.

In rodents, exposure of prepubertal females to an intact (non-castrated) male or its odor accelerates the onset of puberty. This phenomenon is known as the "Vandenbergh effect"[11–13] and is controlled by the olfactory system.[14,15] In goats, it was recently demonstrated that sexually active males also trigger a synchronized early puberty in females (about 1.5 months).[16] This effect has been revealed by hormonal variations of luteinizing hormone (LH) and progesterone as well as enhanced maturation of the reproductive tract.[16] However, the neuroendocrine mechanisms produced by buck exposure causing early puberty have not been clearly identified. For example, no clear differences in the number of cells and density of immunoreactive fibers in the GnRH neural network in the preoptic area (POA) and the arcuate nucleus (ARC) were observed between pubescent females exposed to sexually active males and non-pubescent isolated females.[16] However, the kisspeptin neuronal network in these regions has not been characterized in the context of male-induced puberty.

Kisspeptin is a neuropeptide of the RF-amide family encoded by the *Kiss1* gene that binds to the GPR54/KISS1R receptor especially to stimulate GnRH release.[17,18] Two anatomically distinct populations of kisspeptin neurons have been identified. In rodents, one population is found in the ARC and the other in the rostral part of the hypothalamus, the anteroventral periventricular nucleus (AVPV) as well as the medial POA (mPOA) in non-rodent mammals.[19–21] In rodents, the ARC population has been proposed to mediate the estradiol (E2) negative feedback on GnRH/LH secretion, whereas the AVPV population is thought to be responsible for the E2 positive feedback on GnRH/LH surge.[20,22] While the location of the LH surge generator in ewes remains debated with the involvement of one or both populations,[23–26] some data suggest the involvement in does of only mPOA kisspeptin neurons and not ARC neurons.[27]

In mammals, kisspeptin neurons are now considered the gatekeepers of pubertal transition (see review[28]). For example, chronic central administration of kisspeptin to prepubescent rats advances the time of vaginal opening.[29] In sheep, repeated intravenous injections of kisspeptin in prepubescent ewe lambs stimulate LH secretion until the preovulatory peak leading to ovulation.[30] In the latter case, luteal activity following ovulation was limited and estrous cycles were not maintained in the absence of treatment. These results support an important role for kisspeptin in the maturation of the reproductive axis responsible for the onset of puberty.[29,30] In various species including the goat, ARC kisspeptin neurons coexpress neurokinin B (NKB) and dynorphin A (Dyn) [KNDy neurons[31,32]], which are known to promote and inhibit GnRH secretion respectively. A study conducted in ewes showed an increase in kisspeptin-immunoreactive cells and NKB fiber immunoreactivity in the ARC of pubescent (>9 months) compared to prepubescent females (5–6 months),[33] suggesting that kisspeptin and potentially NKB regulate the ovine pubertal transition.[33]

In the study of Chasles et al.[16] bucks introduced just after weaning of the females at the end of June induce female puberty in early September, when the intact males became sexually active. Here, we studied the effect of a shorter exposure (from mid-August) of females to bucks on early puberty. We hypothesized that male sexual activity is a key factor in this phenomenon, and that this shortened period would be sufficient to induce earlier puberty in exposed females. We then studied the effect of buck exposure on the early maturation of the kisspeptin neural network, in the context of pubertal transition.

2 | METHODS

2.1 | Animals

Experiments were performed from June to October 2021 in Nouzilly, France (latitude 47° 32 N and longitude 0° 46E) on Alpine goats (*Capra hircus*). We used 46 animals for this study: 38 prepubescent does and eight sexually experienced adult bucks. Does were born in 2021, between January 15 and January 25 and weaned at 2.5 months of age. One week before the start of the experiment, females were allocated into four groups balanced for bodyweight. Littermates were separated to avoid genetic effects. Animals were fed daily with hay, straw and commercial pellets with free access to water and mineral blocks. Females were weighed once a month to ensure that the average weight between groups was similar and to detect a potential metabolic effect.

All procedures were performed following European directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the local ethics committee for animal experimentation (CEEA VdL, Tours, France, n°2021040111221610 and n°2021051909002944).

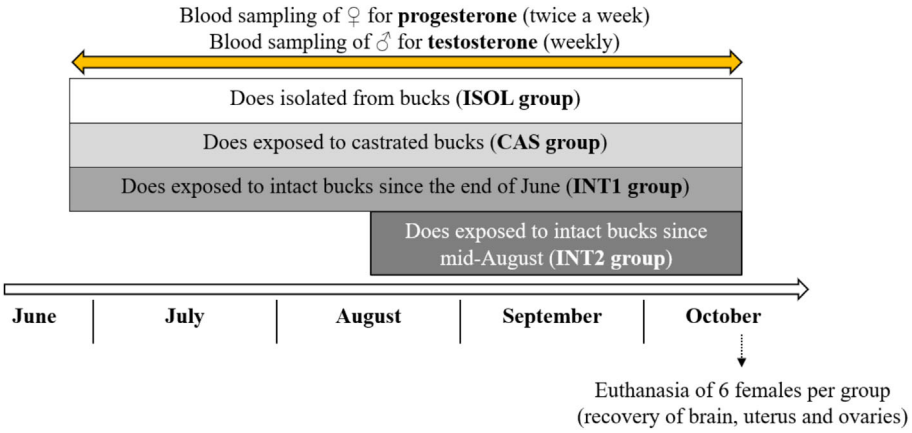

FIGURE 1 Experimental diagram. From June 28, females were divided into four groups: one isolated from bucks (ISOL, $n = 8$), one exposed to wethers (CAS, $n = 10$), one exposed to intact bucks from the end of June (INT1, $n = 10$) and one joining the intact bucks only in mid-August (INT2, $n = 10$). In the CAS, INT1 and INT2 groups, stimulation was continuous with males separated from females by a barrier. Twice a week, a buck was introduced for 1 h into the females' pen to allow observation of sexual behavior. Six females from each group were euthanized in mid-October to recover the brain, uterus and ovaries.

2.2 | Experiment 1. Limiting the exposure period of prepubescent does to the sexual activity period of males

2.2.1 | Exposure to bucks

From June 28 until October 17, one group of females was isolated from males (ISOL, $n = 8$), a second group was exposed to wethers (CAS, $n = 10$), and a third group to intact bucks (INT1, $n = 10$), following the protocol of Chasles et al.[16] A fourth group was isolated from males at the beginning of the experiment and then exposed to intact bucks from August 16 (INT2, $n = 10$; Figure 1). This timing of introduction was chosen to allow at least 2 weeks of cohabitation next to the males before these males became sexually active in early September,[16] and avoid carrying out the usual "male effect" protocol.[9] Doe groups were housed in different barns to prevent odor contamination (distance >300 m). Bucks of the CAS group ($n = 4$) were castrated under ketamine/xylazine (Ketamidor/ Rompun) and isoflurane (Isoflurin) anesthesia 1 month before the experiment. Intact bucks ($n = 4$) were used for both INT1 and INT2 groups.

Does from CAS, INT1 and INT2 groups were continuously exposed to the bucks located in an adjacent pen (4 males for 8–10 females) separated by a fence and allowing direct contact between sexes. Twice a week in all exposed groups, one male fitted with an apron was introduced for 1 h into the female enclosure to allow direct contact and behavioral teasing. Bucks were weekly switched to prevent possible habituation and interindividual effects.

2.2.2 | Assessment of male sexual activity

To evaluate male sexual activity during the experiment, blood samples were collected by jugular venipuncture twice a month to measure testosterone concentration. A male was considered sexually active when testosterone levels exceeded 10 ng/mL.[16] Testicular weight and odor intensity were also considered as markers of male sexual status. Testicular weight was estimated every 2 weeks by appreciation of testicular volume via comparative palpation with an orchidometer.[34] Odor was subjectively evaluated every week using the method of Walkden-Brown et al.[35] In brief, all bucks were smelled about 10 cm from the neck and allocated a score of 0 (neutral odor), 1 (weak male odor), 2 (moderate male odor) or 3 (strong male odor). To avoid perception bias, the same operator carried out these measurements during the whole experiment.

Buck behavior was observed during the introduction to the female enclosure, measuring the number of male/female physical contacts, anogenital sniffing, flehmens, lateral approaches, and mounts.[10,16,36]

2.2.3 | Evaluation of puberty onset

Age at puberty was determined by assessing the onset of ovulatory activity. Twice a week, blood samples were collected in 5 mL heparinized tubes by jugular venipuncture from all females starting from June 28 to assess progesterone levels. Ovulation was considered to have occurred when progesterone concentrations were ≥1 ng/mL in two consecutive samples.[37] At this point, females were considered pubescent.

2.3 | Experiment 2. Impact of exposure to sexually active bucks on the reproductive tract maturation and the kisspeptin neuroendocrine network of does

2.3.1 | Euthanasia and perfusion

When all INT1 does had reached puberty at 264 days of age (D264) according to the biweekly progesterone samples, six females per

group were randomly selected to compare the maturation of neuroendocrine networks involved in GnRH secretion and to verify the development of the reproductive tract. Females were anesthetized with intravenous injection of 5 mL ketamine (Ketamidor) then euthanized by a licensed butcher between October 18 and 21. Blood samples were taken from all females on the day of euthanasia for progesterone assay.

Heads were immediately perfused through both carotid arteries with 2 L of 1% sodium nitrite and 4 L of 4% paraformaldehyde solution. The brain was removed from the skull following perfusion and then cut into blocks. Blocks were post-fixed for 24 h in the same fixative and transferred to a 20% sucrose solution until sinking. Coronal sections of the hypothalamus-containing block (30 µm thickness) were performed with a cryomicrotome (Leica) and then stored in PBS-azide buffer at 4°C.

Uteri and ovaries were fixed for 24 h by immersion in 4% paraformaldehyde and then stored in a 20% sucrose solution at 4°C. After cleaning, uteri and ovaries were weighed, and the number of corpora lutea counted.

2.3.2 | Kisspeptin immunohistochemistry

Immunolabeling was performed on slices from the mPOA as well as the medial and caudal parts of the ARC (ARCm and ARCc) according to the Sheep Brain Atlas of the Brain Biodiversity Bank at Michigan State University. These regions are known to contain kisspeptin neurons in goats.[27,31,38] Three sections of each region of interest were chosen from each doe to perform the immunostaining. Free-floating sections were rinsed three times for 5 min in PBS and then preincubated for 1 h with PBS-0.1% Triton X-100 (PBST) with 2% NDS (normal donkey serum) and 1% BSA (bovine serum albumin). Next, sections were incubated for 48 h at 4°C in PBST 0.1-NDS 2%-BSA 1% with a primary rabbit anti-kisspeptin antibody (1:10000, AC566, RRID: AB_2622231), already used for this species.[39] After three 5 min washes in PBST 0.1, sections were incubated 2 h at room temperature in PBST 0.1 with a secondary donkey anti-rabbit Cy3 antibody (1:2000, Jackson ImmunoResearch, RRID: AB_2307443). Sections were then rewashed three times for 5 min in PBS before incubation in a Hoechst solution (1:5000 in PBS for 2 min) to label the cell nuclei. Finally, sections were mounted in PBS, dried, and coverslipped in fluoromount.

2.3.3 | Image analysis and quantification

Sections were examined using a ZEISS Axio Scan.Z1 slide scanner with appropriate filters and using Zen software (Zeiss). Images were acquired at ×20 magnification by performing a z-stack followed by the extended depth of focus feature. ARC sections were analyzed using QuPath software (version 0.3.1). The area of kisspeptin-immunoreactive fibers was determined by applying a suitable threshold classifying the pixels in the region of interest. The fiber density was calculated by dividing the immunoreactive detection area by the total surface of the study region. Kisspeptin-immunoreactive cell bodies were counted by detecting cells with Hoechst-stained nuclei combined with an adequate threshold of Cy3 labeling. mPOA sections were analyzed using ImageJ (version 2.9.0, Fiji) software. The triangle thresholding algorithm of ImageJ was used to correct the heterogeneous background noise between the different images (https://imagej.net/plugins/auto-threshold). Fibers' density was also calculated on mPOA sections.

2.4 | Hormone assays

Blood samples were centrifuged for 30 min at 4000 *g* to collect plasma. For males, plasma testosterone concentration was determined by radioimmunoassay[40] with a sensitivity of 0.1 ng/mL. For females, plasma progesterone concentration was determined by enzyme immunoassay with a sensitivity of 0.25 ng/mL.[41] The intra-and inter-assay coefficients of variation were <9% and <8.7%, respectively.

2.5 | Statistical analysis

Data are reported as mean ± SEM (standard error of the mean). Statistical tests were performed using Rstudio software (R version 4.1.0, "nparcomp" package). A Shapiro–Wilk test examined the normality of the data distribution. Parametric tests were performed if the data were normally distributed ($p > .05$) and non-parametric tests if this condition was not respected. Homoscedasticity (equality of variances) was also checked to use the appropriate statistical test.

The same intact males (INT) were used to stimulate females in the INT1 and INT2 groups. Thus, buck behavior was compared by unpaired (CAS vs. INT1 and INT2) or paired (INT1 vs. INT2) Wilcoxon rank sum tests. Age at puberty was evaluated using Kaplan–Meier survival analysis and was compared using the log-rank test. The area under the curve (AUC) was assessed to compare the cumulative plasma progesterone secreted with a one-way ANOVA test with heterogeneous variances followed by a Games-Howell post hoc test. Uterus and ovary weights were compared using a one-way ANOVA test followed by Tukey HSD post hoc analyses. The proportion of does presenting corpora lutea was compared using Fisher's exact test. The number of kisspeptin-immunoreactive cell bodies was analyzed using a Kruskal-Wallis test followed by Dunn post hoc analyses. Kisspeptin fiber density was examined using a one-way ANOVA test followed by Tukey HSD post hoc analyses.

In order to maximize the effect of the male induced puberty on the kisspeptin network, regardless of the time of exposure to sexually active males, we chose to pool the two groups exposed to intact males INT1 and INT2 (pool EXPO) and the two control groups ISOL and CAS (pool CTRL). This resulted in no significant difference being detected between the two groups in the same pool and was done in accordance to the use of biological reasoning rather than statistical rules as stated in Balthazart and McCormick.[42] ARC fiber density and

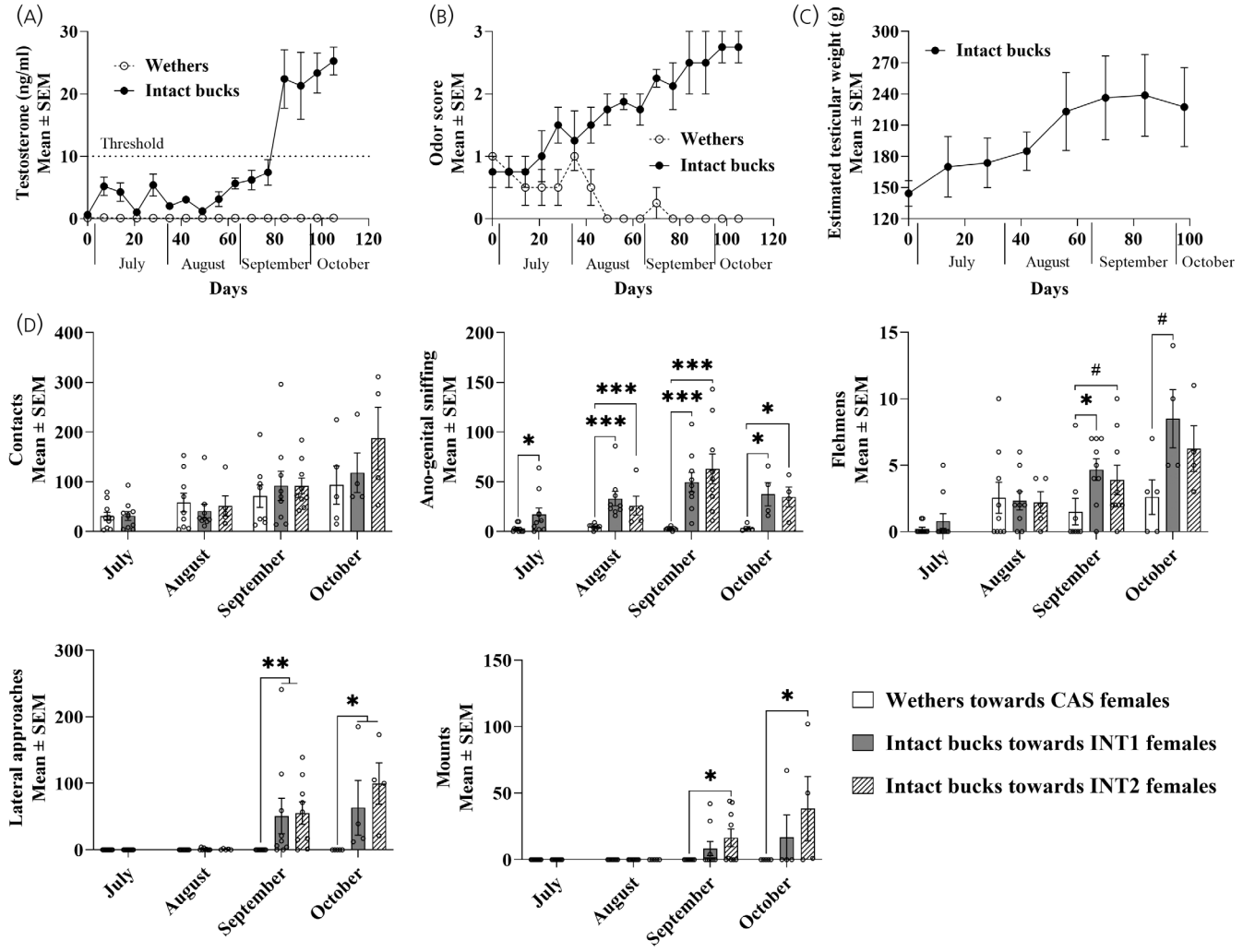

FIGURE 2 Assessment of male sexual activity between the intact bucks ($n = 4$) compared to the wethers ($n = 4$). Data are represented as mean ± SEM. (A) Testosterone levels were assessed once a week. Values above the threshold of 10 ng/mL were indicative of entry into the breeding period of the bucks. (B) An odor score was made weekly by assigning 0 (neutral odor), 1 (weak male odor), 2 (moderate male odor) or 3 (strong male odor). (C) Testicular weight was estimated every fortnight. (D) Sexual behavior of the bucks was assessed during each introduction (1 h, twice a week). Buck behavior was analyzed by unpaired (wethers towards CAS females vs. intact bucks towards INT1 or INT2 females) or paired (intact bucks towards INT1 females vs. intact bucks towards INT2 females) Wilcoxon rank sum tests (#.05 < p < .07, *p < .05, **p < .01, ***p < .001).

the number of kisspeptin-immunoreactive cell bodies were analyzed with Wilcoxon rank sum tests. Fiber density of mPOA was examined using a *t*-test.

3 | RESULTS

3.1 | Male sexual activity

Testosterone levels in all males increased during the experiment, rising above the 10 ng/mL threshold in mid-September, except for wethers (Figure 2A, Figure S2A). Odor scoring and testicular weight also increased in intact males (Figure 2B,C, Figure S2B). In contrast, the odor score of wethers decreased after castration.

Concerning male sexual behavior (Figure 2D), we found no significant differences between groups in terms of direct contact. However, intact males displayed more anogenital sniffing than wethers (July: CAS vs. INT1, $W = 21$, $p < .05$; August–September: CAS vs. INT1 and CAS vs. INT2, $W = 0$, $p < .001$; October: CAS vs. INT1, $W = 0$, CAS vs. INT2, $W = 0.5$, $p < .05$). A significant increase ($W = 14.5$, $p < .05$) of flehmen behavior was observed only in September in intact males towards INT1 females compared to wethers. A similar trend was observed between intact males towards INT2 group and wethers in September ($W = 16.5$, $p = .0597$), as well as between intact males towards INT1 group and wethers in October ($W = 2$, $p = .0628$). From September onwards, a significant increase in lateral approaches was observed in intact males to both groups of exposed females compared to wethers (September: CAS vs. INT1, W = 8, CAS vs. INT2,

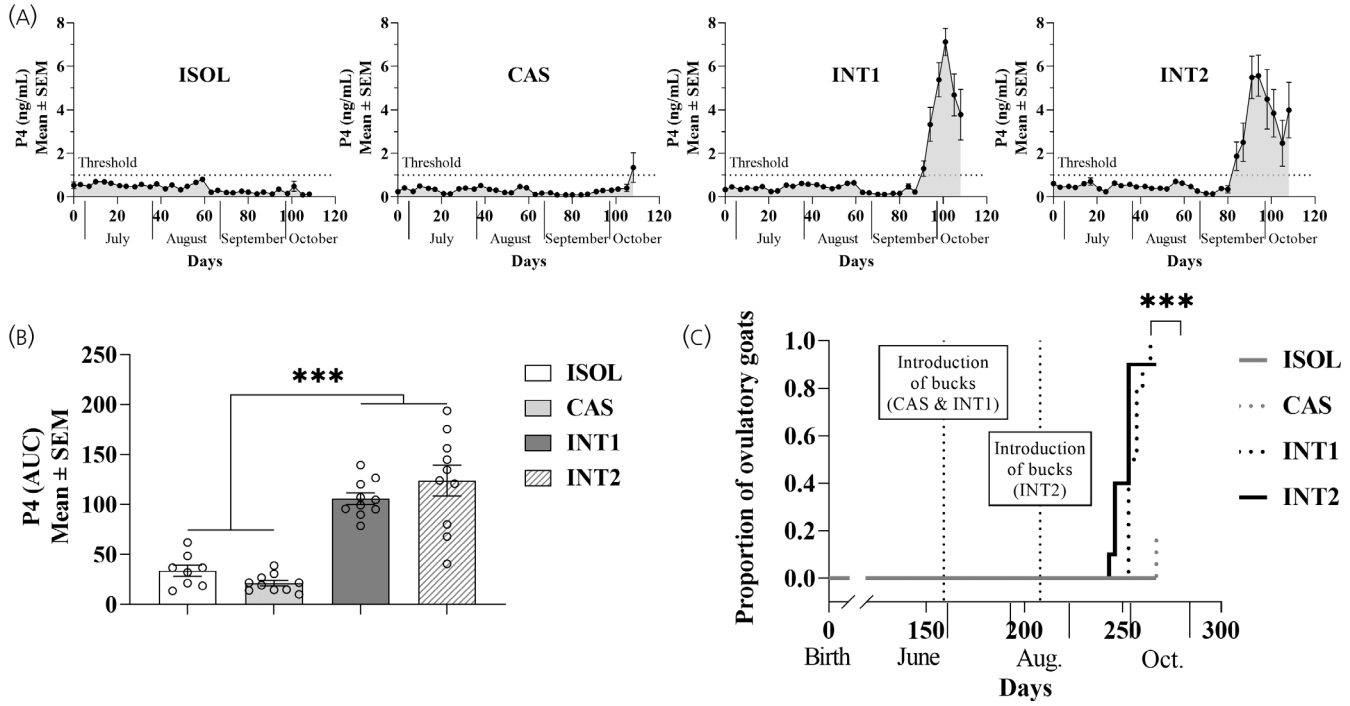

FIGURE 3 Variations in progesterone secretion levels in young does. From June 28, one group of females was isolated from males (ISOL, $n = 8$), one was exposed to wethers (CAS, $n = 10$), one directly exposed to intact males (INT1, $n = 10$) and one group remained isolated before joining the intact males from August 16 (INT2, $n = 10$). Blood samples were collected twice a week. (A) Representative mean profiles of plasma progesterone secretion for each group. (B) Comparison of cumulative progesterone levels per group represented by the area under the curve (AUC). Data are represented as mean ± SEM and compared by one-way ANOVA (heterogeneous variances) followed by Games-Howell post hoc analyses (***$p < .001$) (C) Proportion of ovulating does. Females were considered pubescent when two consecutive progesterone values were ≥1 ng/mL. The log-rank test was used to compare the groups (***$p < .001$).

W = 4, $p < .01$; October: CAS vs. INT1 and CAS vs. INT2, $W = 0$, $p < .05$). Only intact males towards INT2 group displayed significantly higher mounting behavior in September and October when compared to wethers (September: $W = 16$; October: $W = 2.5$, $p < .05$ in both months). No significant differences were observed in the behavioral displays of intact males towards INT1 and INT2 females throughout the experiment.

3.2 | Experiment 1. Shortening of the exposure period of prepubescent does to sexually active bucks

There was no weight difference between groups during the experiment, except in August between the CAS and INT1 groups (Figure S1). Females in the groups exposed to intact males (INT1 and INT2) ovulated during the experiment measured by an increase of plasma progesterone concentrations (Figure 3A). After calculation of the AUCs, a significant increase was observed in both groups of INT1 and INT2 females compared to the ISOL and CAS groups (ANOVA with heterogeneous variances: $F_{3,\ 16.686} = 63.03$, $p < .001$; post hoc Games-Howell test: $p < .001$, Figure 3B).

We observed that puberty onset differed between groups of females (Figure 3C). Females exposed to intact bucks (INT1 and INT2) had the first ovulation earlier than females exposed to wethers or isolated ones (log-rank test: $\chi 2 = 36.39$, $df = 3$, $p < .001$). No significant differences were observed between the groups of females exposed to intact males (INT1 vs. INT2) and between control groups (ISOL vs. CAS). Half of the does exposed to intact bucks in INT1 and INT2 groups were pubescent at 253 days after birth (D253) whereas this ratio was never achieved in the ISOL and CAS groups. Moreover, all females in both INT groups (except one) had their first ovulation within a 10–11 day interval after the beginning of male breeding season. On the day of sacrifice (D267), nearly all of the females exposed to intact bucks (100% INT1 and 90% INT2) were pubescent. In contrast, 0% ISOL and 20% CAS females exposed to wethers or unexposed were pubescent.

3.3 | Experiment 2. Impact of exposure to sexually active bucks on the reproductive tract maturation and the kisspeptin neuroendocrine network of females

3.3.1 | Reproductive tract maturation

We observed significantly higher uterine weight relative to body-weight in INT1 females compared to wethers-exposed females (ANOVA: $F_{3,\ 20} = 4.296$, $p < .05$; post hoc Tukey HSD test: $p < .05$, Figure 4A,C). A similar trend was also observed with isolated females

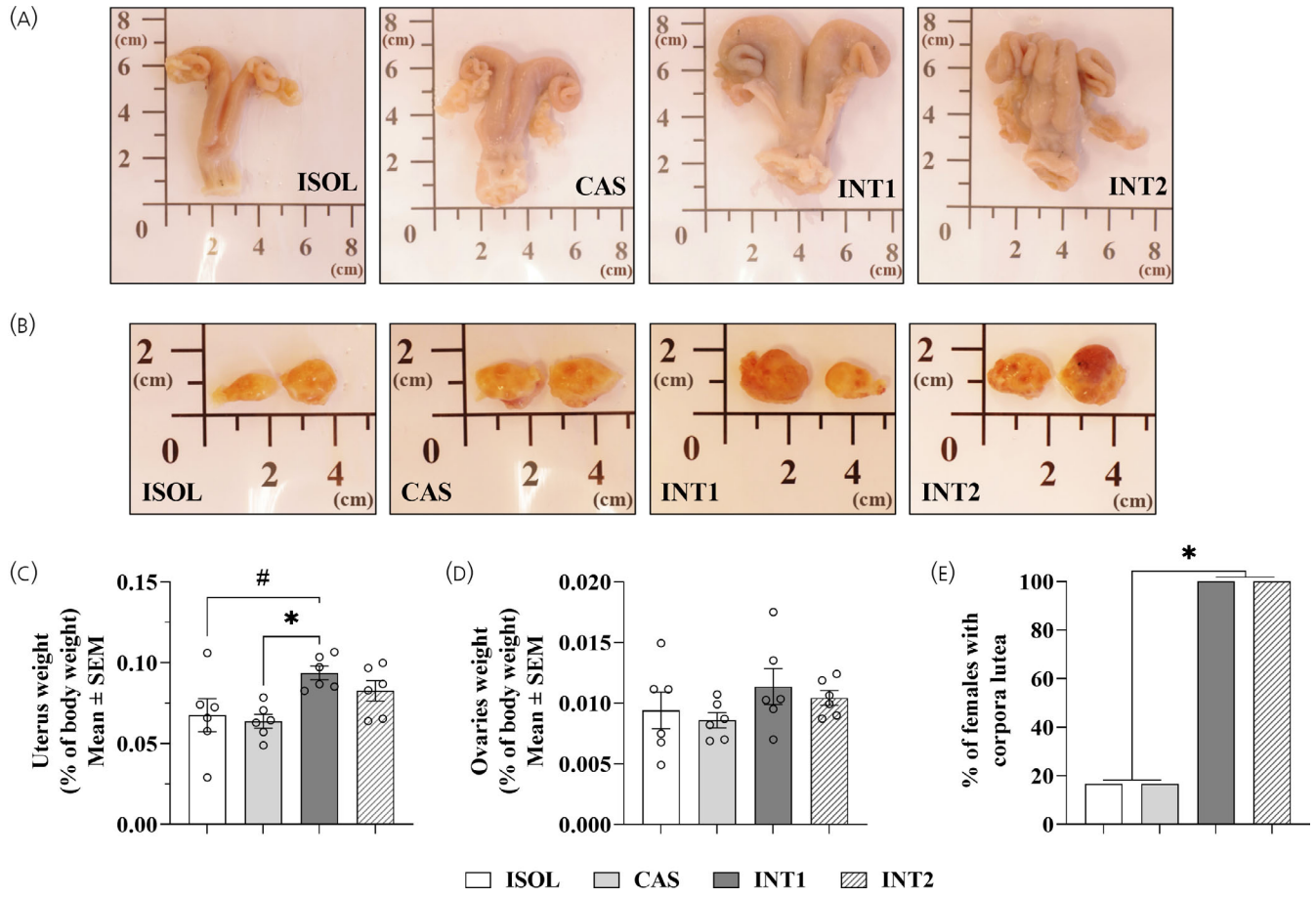

FIGURE 4 Maturation of the reproductive tract by exposure to sexually active bucks. Between October 18 and 21 when all INT1 does had reached puberty, six females per group were randomly selected then euthanized to recover brain, uterus and ovaries. Representative photographs of the uterus (A) and ovaries (B) from ISOL, CAS, INT1 and INT2 females. Graphs representing the comparison of uterine (C) and ovaries (D) weight between ISOL, CAS, INT1 and INT2 groups. Data are shown as mean ± SEM. The comparison was performed by a one-way ANOVA followed by Tukey HSD post hoc analyses (#$p = .053$, *$p < .05$). (E) Comparison of the proportion of females exhibiting corpora lutea. Significant differences between groups were evaluated by Fisher's exact tests (*$p < .05$, ***$p < .001$).

(ANOVA: $F_{3,\ 20} = 4.296$, $p < .05$; post hoc Tukey HSD test: $p = .053$). No significant differences were observed in ovarian weights relative to bodyweight for any of the groups (ANOVA: $F_{3,\ 20} = 1.118$, $p > .05$, Figure 4B,D). Corpora lutea were found in all INT1 and INT2 females, whereas only in one out of six females in the other two groups, ISOL and CAS (Fisher's exact test, $p < .05$, Figure 4E). The only ISOL female exhibiting a corpora lutea showed a progesterone value >1 ng/mL on the day of euthanasia but was not considered to be pubescent because did not show two consecutive high values in the biweekly hormonal monitoring. Importantly, all pubescent INT1 and INT2 females were on the luteal phase according to progesterone levels on the day of euthanasia.

3.3.2 | Development of the kisspeptin neural network

We performed a group by group comparison and found that the mean kisspeptin fiber density in the ARCc was higher in INT1 and INT2 females when compared to CAS females (ANOVA: $F_{3,\ 19} = 6.617$, $p < .01$; post hoc Tukey HSD test: $p < .05$, Figure 5A,B). A similar trend was observed with ISOL females (ANOVA: $F_{3,\ 19} = 6.617$, $p < .01$; post hoc Tukey HSD test: vs. INT1: $p = .063$, vs. INT2: $p = .053$). EXPO females had a significantly higher density of kisspeptin fibers in the ARCc ($W = 9$, $p < .001$) compared to CTRL females. ARCm analysis revealed no significant differences between the four groups, although fiber density tended to be higher in EXPO vs. CTRL females ($W = 34$, $p = .051$). The number of kisspeptin-immunoreactive cell bodies in INT1 and INT2 females was significantly higher than CAS females in the ARCc (Kruskal-Wallis: $\chi 2 = 10.57$, $df = 3$, $p < .05$; posthoc Dunn test: $p < .05$, Figure 5C). Similarly, EXPO females showed a larger number of kisspeptin-immunoreactive cell bodies in the ARCc ($W = 18$, $p < .01$).

We next analyzed the mPOA but no significant differences were observed between the four groups (ANOVA: $F_{3,\ 20} = 3.194$, $p = .046$; post hoc Tukey HSD test: $p > .05$, Figure 5D,E). However, the CTRL/EXPO comparison revealed a significantly higher kisspeptin fiber density in CTRL females compared to EXPO females (*t*-test: $t_{22} = 3.123$, $p < .01$).

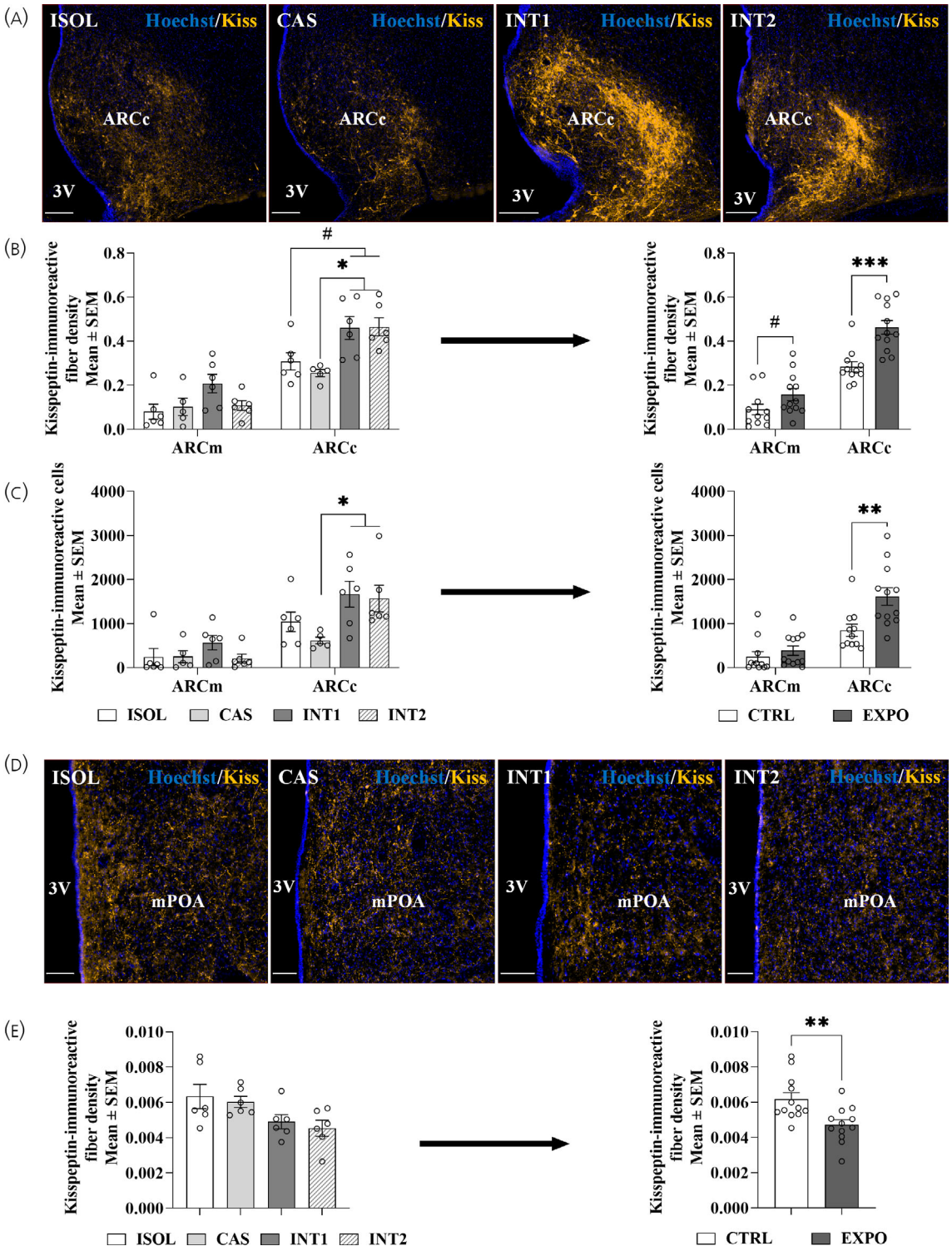

FIGURE 5 Maturation of the kisspeptin neural network by exposure to sexually active bucks. (A) Representative photomicrographs of ISOL, CAS, INT1 and INT2 females for kisspeptin-immunoreactive (−ir) cells and fibers (orange) and nuclear labeling (Hoechst, blue) in caudal ARC. Scale bars, 200 µm. Graphs representing the comparison of (B) kisspeptin-immunoreactive fiber density and (C) kisspeptin immunoreactive cells in ARCm and ARCc. (D) Representative pictures of ISOL, CAS, INT1 and INT2 females for kisspeptin-ir fibers (orange) and nuclear labeling (Hoechst, blue) in mPOA. Scale bars, 100 µm. (E) Graphs representing the comparison of kisspeptin-immunoreactive fiber density in mPOA. Data are represented as mean ± SEM. Comparisons between ISOL, CAS, INT1 and INT2 were performed using (B and E, $n = 5–6$) a one-way ANOVA followed by Tukey HSD post hoc analyses (#$p = .063$ and .053, *$p < .05$) and (C) a Kruskal-Wallis test followed by Dunn post hoc analyses (*$p < .05$). Comparisons between EXPO and CTRL were carried out by (B–C) a Wilcoxon rank sum test (#$p = .051$, **$p < .01$, ***$p < .001$) and (E) a *t*-test (**$p < .01$). 3 V, third ventricle; ARCc, caudal arcuate nucleus; mPOA, medial preoptic area.

We found no significant differences in the fiber density and number of kisspeptin-immun'oreactive cell bodies between INT1 and INT2 females with very early puberty compared to females in the same groups with slightly delayed early puberty (Figure S3).

4 | DISCUSSION

This study shows that short exposure of spring-born females to sexually active males can induce early puberty. This male-induced puberty onset is manifested by an early maturation of the reproductive tract but also by the development of the kisspeptin neural network, more specifically in the ARCc.

The first experiment demonstrates that short exposure to sexually active males from mid-August is sufficient to trigger early pubertal transition in does. These results confirm the efficiency of sexually active bucks in inducing this phenomenon.[16] Interestingly, exposed females began to ovulate when male sexual activity increased around mid-September. The male sexual activity was manifested in an increase of plasma testosterone concentration as well as the expression of typical breeding season behaviors such as lateral approaches and mounting. This activity was not found in our wethers. Furthermore, all females exposed to intact males (except one INT2) became pubescent within 3 weeks of this increase in sexual activity. Thus, these results support the hypothesis that male sexual activity is the main catalyst for early female puberty rather than the physical presence of the male per se. In the context of the male effect, the sexual activity of bucks is the main factor for triggering LH release and ovulation in females during the anestrous period.[10,43–45] In our study, we chose to allow the INT2 group to cohabitate next to the males, before they entered the breeding season, for approximately 2 weeks. This cohabitation period was implemented to avoid a conventional "male effect" protocol that could induce an acute effect (see review[9]). This introduction timing (mid-August) was also chosen because intact males typically enter the breeding season in early September.[16] Yet during the experiment, intact males became sexually active in mid-September, thus extending this habituation period to 1 month. The reasons for this delay remain unclear, but different summer climate than in previous years may have played a role. Taken together, our results suggest that exposure (cohabitation) period can be further reduced to induce early puberty in females.

The second experiment shows that short exposure induces early maturation of the reproductive tract as did prolonged exposure. Our results confirm that this exposure to sexually active males is responsible for this maturation.[16] Indeed, 100% of the females exposed to sexually active males displayed at least one corpora lutea, indicating ovulatory activity. Uterine weight was, on average, 30% higher in the INT1 group vs. CAS group. As in the ewe,[46] the increase in uterine weight is probably caused by a change in sensitivity to ovarian steroids during the pubertal transition. To our knowledge, no study has yet evaluated zootechnical parameters such as fertility, prolificacy, kidding and productivity of does during breeding following an early puberty protocol under natural conditions (without using treated bucks or exogenous progesterone), except under semi-extensive management conditions.[47]

The number of kisspeptin neurons and fiber density were higher in the ARCc of pubescent does exposed to sexually active males compared to non-pubescent unexposed females. In sheep, the number of kisspeptin neurons was higher in the ARC of ovary-intact pubescent ewes during the early follicular phase (>9 months) compared to prepubescent ewes (5–6 months).[33] However, no such difference was found during pubertal development in E2-treated ovariectomized ewes (OVX + E2), both at the level of *Kiss1* transcripts[48,49] and kisspeptin immunoreactivity.[49] The choice of conditions (ovary-intact vs. OVX + E2 females) could explain the divergence of these outcomes.[49] Our results in ovary-intact pubescent does during the luteal phase are consistent with the observations of Nestor et al.[33] Although in female goats the number of kisspeptin-immunoreactive neurons seems to be greater during the follicular phase than during the luteal phase,[50] the kisspeptin neuronal network may be sufficiently developed to maintain cyclic ovarian activity in pubescent females exposed to sexually active males. Thus, our results support the hypothesis of a key role for ARC kisspeptin in the caprine female pubertal transition. The shift previously reported in ovary-intact ewes corresponds to a period of at least 3–4 months.[33] Here we observe a shift between prepubescent and male-induced pubescent does at the same age, suggesting that sensory stimuli from sexually active bucks induce rapid neuroendocrine changes leading to early puberty onset.

Interestingly, we found differences in the kisspeptin network of ARCc but not of ARCm. The presence of *Kiss1* transcripts appears to be rather similar in ARCm and ARCc.[27] Yet, immunohistochemical approaches show a particular abundance of kisspeptin protein in the caudal part of the ARC.[31,38,50] The approach used in this study could explain why the results obtained in the ARCc are not found in the ARCm.

In several mammalian species, ARC kisspeptin neurons coexpress NKB and DynA (KNDy neurons[32]). In goats, some studies show that NKB is colocalized with most of ARC kisspeptin neurons (97%–99.5%).[31,38] In sheep, NKB-immunoreactive fiber density is greater in the ARC of ovary-intact pubescent ewes during the early follicular phase (>9 months) compared to prepubescent ewes (5–6 months),[33] but not in OVX + E2 females.[49] Nonetheless, NKB could also have a key role during puberty onset. Data from Wakabayashi and coworkers[31,51] support the hypothesis that ARC kisspeptin neurons, specifically KNDy neurons, form a dense and interconnected neural circuit to synchronize their activity enabling pulsatile-like secretion of GnRH. Thus, we hypothesize that the development of the KNDy neural network could be a major neuroendocrine change leading to GnRH secretion and therefore to an earlier pubertal transition induced by the buck.

In the present study, kisspeptin fiber density was reduced in the mPOA of pubescent does exposed to sexually active males compared to control females. As in the study of Nestor et al.[33] we were unable to quantify the cell bodies of the kisspeptin neurons, but we could easily discern their fibers. In the POA of OVX + E2 ewes, *Kiss1* transcripts increased with age during pubertal development.[48] Our results

seem contradictory to the results from the OVX + E2 ewes. This could be explained by a disconnection between *Kiss1* transcripts and the protein and/or our choice of ovary-intact model. Interestingly in adult OVX does, supplementation with high E2 level significantly increases c-Fos expression (marker of neuronal activation) in mPOA *Kiss1* cells suggesting their involvement in the induction of LH surge.[27] It is important to note that all females exposed to sexually active bucks were euthanized during the luteal phase. Thus, we speculate that the density of kisspeptin-immunoreactive fibers could be lowered during the luteal phase following the LH surge.

Repeated injections of kisspeptin stimulate LH secretion until the preovulatory peak leading to ovulation in prepubescent ewes.[30] Yet, regular estrous cycles cannot be maintained without treatment indicating that the neuroendocrine axis of reproduction is not fully mature.[30] Interestingly, cyclic ovarian activity of does is maintained after male-induced puberty onset[16,52] probably via the early maturation of the ARC kisspeptin network (this study). The induced early maturation of this kisspeptin network likely underlies the maturation of other upstream networks that would be interesting to explore.

In the context of the male effect, introduction of rams to anestrous ewes activates ARC kisspeptin neurons leading to increased GnRH/LH secretion.[53] This does not seem to be the case in goats.[39] Thus, whether the detection of male sociosexual signals is transduced to ARC kisspeptin neurons in goats remain unclear. Interestingly, buck olfactory cues have been shown to trigger the hypothalamic GnRH pulse generator using by recording multiple-unit activity,[54,55] in the region of the ARC KNDy neurons.[56–58] In sheep, the ram pheromone signal seems to be transduced via the medial nucleus of the amygdala to KNDy neurons, thereby stimulating GnRH secretion in the ewe.[57] Moreover, buck odor is known to induce a short-term effect (LH secretion[45]) that can lead to a long-term effect (ovulation[59,60]:). Therefore, although we cannot exclude the participation of visual and acoustic cues, olfactory signals could play an important role in the precocious puberty effect in does.

In summary, our results show that a short exposure to sexually active bucks is sufficient to induce doe precocious puberty. Thus, exposure to bucks while they are inactive seem unnecessary to trigger female puberty onset. This study also demonstrates that exposure of prepubertal females to sexually active males induces neuroendocrine changes, particularly in the kisspeptin neuronal network of the ARCc. Since kisspeptin has a key role in the pubertal transition, these changes may be responsible for the early initiation of ovulatory activity in females. Further research is needed to determine the sensory modalities underlying this early hypothalamic maturation.

AUTHOR CONTRIBUTIONS

Maxime MEUNIER: Conceptualization; formal analysis; investigation; writing – original draft; writing – review and editing. **Chantal Porte:** Investigation; writing – original draft; writing – review and editing. **Kévin Poissenot:** Investigation; writing – original draft; writing – review and editing. **Hélène Vacher:** Investigation; writing – original draft; writing – review and editing. **Morgane Brachet:** Investigation; writing – original draft; writing – review and editing. **Pablo Chamero:** Writing – original draft; writing – review and editing. **Massimiliano Beltramo:** Writing – original draft; writing – review and editing. **José A. Abecia:** Writing – original draft; writing – review and editing. **José A. Delgadillo:** Conceptualization; writing – original draft; writing – review and editing. **Philippe Chemineau:** Conceptualization; writing – original draft; writing – review and editing. **Matthieu Keller:** Conceptualization; funding acquisition; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGMENTS

We thank (1) Olivier Lasserre and all the shepherds of the UEPAO INRAe experimental unit for animals care; (2) Gilles Gomot and the staff of the PIXANIM platform for castration procedures; (3) Sylvain Bourgeais and Benoit Marteau for euthanizing the animals; (4) Anne-Lyse Lainé and the staff of the endocrinology laboratory for progesterone and testosterone measurements; (5) Marie-Claire Blache of the PIC platform for her support in image analysis and the use of QuPath and ImageJ software.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jne.13284.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

FINANCIAL INFORMATION

This work was supported by a grant from Région Centre Val de Loire (France) and INRAE (France).

ORCID

Maxime A. Meunier https://orcid.org/0000-0002-0803-0322
Chantal Porte https://orcid.org/0000-0002-8356-4973
Kévin Poissenot https://orcid.org/0000-0003-4236-1421
Hélène Vacher https://orcid.org/0000-0001-7853-7156
Morgane Brachet https://orcid.org/0000-0002-0911-3744
Pablo Chamero https://orcid.org/0000-0002-4305-1073
Massimiliano Beltramo https://orcid.org/0000-0003-3472-2477
José A. Abecia https://orcid.org/0000-0003-2827-3054
José A. Delgadillo https://orcid.org/0000-0003-4444-188X
Philippe Chemineau https://orcid.org/0000-0002-1584-7285
Matthieu Keller https://orcid.org/0000-0002-5445-7431

REFERENCES

1. Ebling FJP. The neuroendocrine timing of puberty. *Reproduction*. 2005;129:675-683.
2. Herbison AE. Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. *Nat Rev Endocrinol*. 2016;12:452-466.
3. Chemineau P, Daveau A, Maurice F, Delgadillo JA. Seasonality of estrus and ovulation is not modified by subjecting female alpine goats to a tropical photoperiod. *Small Rumin Res*. 1992;8:299-312.

4. Foster DL, Hileman SM. Puberty in the sheep. *Knobil and Neill's Physiology of Reproduction*. 2015; Elsevier:1441-1485.
5. Papachristoforou C, Koumas A, Photiou C. Seasonal effects on puberty and reproductive characteristics of female Chios sheep and Damascus goats born in autumn or in February. *Small Rumin Res*. 2000;38:9-15.
6. Delgadillo JA, Hernández H, Abecia JA, Keller M, Chemineau P. Is it time to reconsider the relative weight of sociosexual relationships compared with photoperiod in the control of reproduction of small ruminant females? *Domest Anim Endocrinol*. 2020;73:106468.
7. Underwood E, Shier F, Davenport N. Studies in sheep husbandry in Western Australia. V. the breeding season in merino, crossbreed and British breed ewes in the agricultural districts. *J Agric West Aust*. 1944;21:135-143.
8. Shelton M. Influence of the presence of a male goat on the initiation of estrous cycling and ovulation of angora does. *J Anim Sci*. 1960;19:368-375.
9. Chemineau P. Possibilities for using bucks to stimulate ovarian and oestrous cycles in anovulatory goats—a review. *Livest Prod Sci*. 1987;17:135-147.
10. Flores JA, Véliz FG, Pérez-Villanueva JA, et al. Male reproductive condition is the limiting factor of efficiency in the male effect during seasonal anestrus in female goats. *Biol Reprod*. 2000;62:1409-1414.
11. Vandenbergh JG. Effect of the presence of a male on the sexual maturation of female mice. *Endocrinology*. 1967;81:345-349.
12. Vandenbergh JG. Male odor accelerates female sexual maturation in mice. *Endocrinology*. 1969;84:658-660.
13. Vandenbergh JG. Acceleration of sexual maturation in female rats by male stimulation. *J Reprod Fertil*. 1976;46:451-453.
14. Flanagan KA, Webb W, Stowers L. Analysis of male pheromones that accelerate female reproductive organ development. *PLoS One*. 2011;6:e16660.
15. Oboti L, Pérez-Gómez A, Keller M, et al. A wide range of pheromone-stimulated sexual and reproductive behaviors in female mice depend on G protein Gαo. *BMC Biol*. 2014;12:1-17.
16. Chasles M, Chesneau D, Moussu C, et al. Sexually active bucks are a critical social cue that activates the gonadotrope axis and early puberty onset in does. *Horm Behav*. 2018;106:81-92.
17. Ohtaki T, Shintani Y, Honda S, et al. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*. 2001;411:613-617.
18. Messager S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci USA*. 2005;102:1761-1766.
19. Franceschini I, Lomet D, Cateau M, Delsol G, Tillet Y, Caraty A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. *Neurosci Lett*. 2006;401:225-230.
20. Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology*. 2005;146:3686-3692.
21. Francia S, Pifferi S, Menini A, et al. *Vomeronasal Receptors and Signal Transduction in the Vomeronasal Organ of Mammals*. http://www.ncbi.nlm.nih.gov/pubmed/24830038 2014.
22. Smith JT, Clay CM, Caraty A, Clarke IJ. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. *Endocrinology*. 2007;148:1150-1157.
23. Estrada KM, Clay CM, Pompolo S, Smith JT, Clarke IJ. Elevated KiSS-1 expression in the arcuate nucleus prior to the cyclic preovulatory gonadotrophin-releasing hormone/lutenising hormone surge in the ewe suggests a stimulatory role for kisspeptin in oestrogen-positive feedback. *J Neuroendocrinol*. 2006;18:806-809.
24. Smith JT, Li Q, Pereira A, Clarke IJ. Kisspeptin neurons in the ovine arcuate nucleus and preoptic area are involved in the preovulatory luteinizing hormone surge. *Endocrinology*. 2009;150:5530-5538.
25. Hoffman GE, Le WW, Franceschini I, et al. Expression of fos and in vivo median eminence release of LHRH identifies an active role for preoptic area kisspeptin neurons in synchronized surges of LH and LHRH in the ewe. *Endocrinology*. 2011;152:214-222.
26. Merkley CM, Porter KL, Coolen LM, et al. KNDy (kisspeptin/neurokinin B/dynorphin) neurons are activated during both pulsatile and surge secretion of LH in the ewe. *Endocrinology*. 2012;153:5406-5414.
27. Matsuda F, Nakatsukasa K, Suetomi Y, et al. The Luteinising hormone surge-generating system is functional in male goats as in females: involvement of Kisspeptin Neurones in the medial preoptic area. *J Neuroendocrinol*. 2015;27:57-65.
28. Uenoyama Y, Inoue N, Nakamura S, et al. Central mechanism controlling pubertal onset in mammals: a triggering role of kisspeptin. *Front Endocrinol (Lausanne)*. 2019;10:312. doi:10.3389/fendo.2019.00312
29. Navarro VM, Fernández-Fernández R, Castellano JM, et al. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. *J Physiol*. 2004;561:379-386.
30. Redmond JS, Macedo GG, Velez IC, Caraty A, Williams GL, Amstalden M. Kisspeptin activates the hypothalamic-adenohypophyseal-gonadal axis in prepubertal ewe lambs. *Reproduction*. 2011;141:541-548.
31. Wakabayashi Y, Nakada T, Murata K, et al. Neurokinin B and dynorphin a in Kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. *J Neurosci*. 2010;30:3124-3132.
32. Lehman MN, Coolen LM, Goodman RL. Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology*. 2010;151:3479-3489.
33. Nestor CC, Briscoe AMS, Davis SM, Valent M, Goodman RL, Hileman SM. Evidence of a role for kisspeptin and neurokinin B in puberty of female sheep. *Endocrinology*. 2012;153:2756-2765.
34. Delgadillo JA, Leboeuf B, Chemineau P. Abolition of seasonal variations in semen quality and maintenance of sperm fertilizing ability by photoperiodic cycles in goat bucks. *Small Rumin Res*. 1992;9:47-59.
35. Walkden-Brown SW, Restall BJ, Norton BW, Scaramuzzi RJ, Martin GB. Effect of nutrition on seasonal patterns of LH, FSH and testosterone concentration, testicular mass, sebaceous gland volume and odour in Australian cashmere goats. *Reproduction*. 1994;102:351-360.
36. Fabre-Nys C, Gelez H. Sexual behavior in ewes and other domestic ruminants. *Horm Behav*. 2007;52:18-25.
37. Delgadillo JA, Flores JA, Hernández H, et al. Sexually active males prevent the display of seasonal anestrus in female goats. *Horm Behav*. 2015;69:8-15.
38. Okamura H, Yamamura T, Wakabayashi Y. Mapping of KNDy neurons and immunohistochemical analysis of the interaction between KNDy and substance P neural systems in goat. *J Reprod Dev*. 2017;63:571-580.
39. Bedos M, Portillo W, Dubois J-PP, et al. A high level of male sexual activity is necessary for the activation of the medial preoptic area and the arcuate nucleus during the 'male effect' in anestrous goats. *Physiol Behav*. 2016;165:173-178.
40. Hochereau-De Reviers MT, Copin M, Seck M, et al. Stimulation of testosterone production by PMSG injection in the ovine male: effect of breed and age and application to males carrying or not carrying the 'F' Booroola gene. *Anim Reprod Sci*. 1990;23:21-32.
41. Canepa S, Laine A-L, Bluteau A, et al. Validation d'une méthode immunoenzymatique pour le dosage de la progestérone dans le plasma des ovins et des bovins. *Cah Des Tech l'INRA*. 2008;64:19-30.
42. Balthazart J, McCormick C. Statistical rules versus biological reasoning: some apparent conflicts and how to solve them. *Horm Behav*. 2022;137:2021-2023.

43. Véliz FG, Moreno S, Duarte G, et al. Male effect in seasonally anovulatory lactating goats depends on the presence of sexually active bucks, but not estrous females. *Anim Reprod Sci*. 2002;72:197-207.
44. Delgadillo JA, Flores JA, Véliz FG, et al. Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days. *J Anim Sci*. 2002;80:2780-2786.
45. Vielma J, Chemineau P, Poindron P, Malpaux B, Delgadillo JA. Male sexual behavior contributes to the maintenance of high LH pulsatility in anestrous female goats. *Horm Behav*. 2009;56:444-449.
46. Lewis AW, Berardinelli JG. Gross anatomical and histomorphometric characteristics of the oviduct and uterus during the pubertal transition in sheep. *J Anim Sci*. 2001;79:167-175.
47. Espinoza-Flores LA, Andrade-Esparza JD, Hernández H, et al. Male effect using photostimulated bucks and nutritional supplementation advance puberty in goats under semi-extensive management. *Theriogenology*. 2020;143:82-87.
48. Redmond JS, Baez-Sandoval GM, Spell KM, et al. Developmental changes in hypothalamic Kiss1 expression during activation of the pulsatile release of Luteinising hormone in maturing ewe lambs. *J Neuroendocrinol*. 2011;23:815-822.
49. Aerts EG, Harlow K, Griesgraber MJ, et al. Kisspeptin, neurokinin B, and dynorphin expression during pubertal development in female sheep. *Biology (Basel)*. 2021;10:988.
50. Jafarzadeh Shirazi MR, Zamiri MJ, Salehi MS, et al. Differential expression of RFamide-related peptide, a mammalian gonadotrophin-inhibitory hormone orthologue, and kisspeptin in the hypothalamus of Abadeh ecotype does during breeding and anoestrous seasons. *J Neuroendocrinol*. 2014;26:186-194.
51. Wakabayashi Y, Yamamura T, Sakamoto K, et al. Electrophysiological and morphological evidence for synchronized GnRH pulse generator activity among Kisspeptin/neurokinin B/dynorphin a (KNDy) neurons in goats. *J Reprod Dev*. 2013;59:40-48.
52. Chasles M, Chesneau D, Moussu C, et al. Highly precocious activation of reproductive function in autumn-born goats (Capra hircus) by exposure to sexually active bucks. *Domest Anim Endocrinol*. 2019;68:100-105.
53. De Bond JAP, Li Q, Millar RP, et al. Kisspeptin signaling is required for the luteinizing hormone response in anestrous ewes following the introduction of males. *PLoS One*. 2013;8:1-11.
54. Hamada T, Nakajima M, Takeuchi Y, Mori Y. Pheromone-induced stimulation of hypothalamic gonadotropin-releasing hormone pulse generator in ovariectomized, estrogen-primed goats. *Neuroendocrinology*. 1996;64:313-319.
55. Ichimaru T, Takeuchi Y, Mori Y. Stimulation of the GnRH pulse generator activity by continuous exposure to the male pheromones in the female goat. *J Reprod Dev*. 1999;45:243-248.
56. Murata K, Wakabayashi Y, Sakamoto K, et al. Effects of brief exposure of male pheromone on multiple-unit activity at close proximity to kisspeptin neurons in the goat arcuate nucleus. *J Reprod Dev*. 2011;57:197-202.
57. Sakamoto K, Wakabayashi Y, Yamamura T, et al. A population of Kisspeptin/neurokinin B neurons in the arcuate nucleus may Be the central target of the male effect phenomenon in goats. *PLoS One*. 2013;8:e81017.
58. Murata K, Tamogami S, Itou M, et al. Identification of an olfactory signal molecule that activates the central regulator of reproduction in goats. *Curr Biol*. 2014;24:681-686.
59. Claus R, Over R, Dehnhard M. Effect of male odour on LH secretion and the induction of ovulation in seasonally anoestrous goats. *Anim Reprod Sci*. 1990;22:27-38.
60. Walkden-Brown SW, Restall BJ. The male effect in the Australian cashmere goat. 2. Role of olfactory cues from the male. *Anim Reprod Sci*. 1993;32:55-67.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Meunier MA, Porte C, Poissenot K, et al. Male-induced early puberty correlates with the maturation of arcuate nucleus kisspeptin neurons in does. *J Neuroendocrinol*. 2023;35(5):e13284. doi:10.1111/jne.13284