Animal, page 1 of 8 © The Author(s), 2020. Published by Cambridge University Press on behalf of The Animal Consortium
doi:10.1017/S1751731120001330

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The continuous presence of ewes in estrus in spring influences testicular volume, testicular echogenicity and testosterone concentration, but not LH pulsatility in rams

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(Received 1 February 2020; Accepted 19 May 2020)

*The continuous presence of active male small ruminants prevents seasonal anestrus in females, but evidence of the same mechanism operating from the females to the males is scarce. This study assessed the effects of the continuous presence of ewes in estrus in spring on ram sexual activity, testicular size and echogenicity, and LH and testosterone concentrations. On 1 March, 20 rams were assigned to two groups (*n = *10 each): isolated (**ISO**) from other sheep, or stimulated (**STI**) by 12 ewes, which were separated from the rams by an openwork metal barrier, allowing contact between sexes. Each week, four ewes were induced into estrus by intravaginal sponges. Live weight, scrotal circumference, testicular width (**TW**) and length (**TL**) were recorded at the beginning and at the end of the experiment, and testicular volume (**TV**) was calculated; at the same time, testicular ultrasonography and color Doppler scanning were performed. Blood samples (March to May) were collected once per week for testosterone determinations, and at the end of the experiment, blood samples were collected for 6 h at 20-min intervals for LH analysis. Rams were exposed to four estrous ewes in a serving-capacity test. Scrotal circumference, TW and TL were higher in the STI than in the ISO rams (*$P < 0.05$*) in May, and TV was higher (*$P < 0.05$*) in the STI (391 ± 17 cm^3) than in the ISO rams (354 ± 24 cm^3). In ISO rams, the number of white pixels was higher (*$P < 0.01$*) in May (348 ± 74) than in March (94 ± 21) and differed significantly (*$P < 0.01$*) from that of the STI rams in May (160 ± 33). In ISO rams, the number of grey pixels was higher (*$P < 0.05$*) in May (107 ± 3) than it was in March (99 ± 1). Stimulated and ISO rams did not differ significantly in mean LH plasma concentrations (0.8 ± 0.5 v. 0.9 ± 0.4 ng/ml), LH pulses (2.1 ± 0.5 v. 2.2 ± 0.2) and amplitude (2.0 ± 0.4 v. 3.2 ± 0.7 ng/ml, respectively). Stimulated rams had significantly higher testosterone concentrations than ISO rams from April to the end of the experiment. Stimulated rams performed more (*$P < 0.05$*) mountings with intromission (3.0 ± 0.4) than did ISO rams (1.5 ± 0.5). In conclusion, after 3 months in the continuous presence of ewes in estrus in spring, rams had higher TV and some testicular echogenic parameters were modified than isolated rams. Although exposed rams also had higher levels of testosterone after 2 months in the presence of estrous ewes, their LH pulsatility at the end of the study was not modified.*

Keywords: sheep, socio-sexual signals, anestrus, hormones, testicle

Implications

It has been demonstrated that the continuous presence of active male small ruminants prevents seasonal anestrus in females. Our objective was to confirm whether these mechanisms can operate from the females to the males. After 3 months being housed with estrous ewes, rams had higher testosterone levels and increased their testicular volume than isolated rams, but did not change their LH secretion. It seems that the annual photoperiodic inhibition of sexual activity is more easily overridden by socio-sexual stimuli in female than in male small ruminants because males tend to be more strictly dependent on day length than females.

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Introduction

Reproductive seasonality is the most important factor limiting productivity in sheep farms because this species exhibits breeding activity from summer to winter. Thus, lambing occurs in late winter or early spring, which allows lambs to develop at a time when temperature and food availability are favorable (Ortavant *et al.*, 1985). Although photoperiod is the main environmental factor responsible for influencing seasonal reproduction (Yeates, 1949), socio-sexual interactions between rams and ewes can influence the timing of the onset and end of the breeding season by phenomena known as the 'male effect' and the 'female effect' (Hawken and Martin, 2012). The 'ram effect' involves the reintroduction of males to a flock of ewes in anestrus that had been isolated from rams (Martin *et al.*, 1986) and is the most well-studied socio-sexual factor for inducing ovulation in sheep in the seasonal anestrus. Although the odor signals from ram pheromones are the main stimulus in the ram effect (Knight and Lynch, 1980), the behavior of the rams is a key factor in ensuring the best response of ewes to the introduction of rams (Perkins and Fitzgerald, 1994). For example, more ewes that had been exposed to light-induced sexual-activated rams in spring became pregnant and produced more lambs per ewe than did ewes that had been exposed to non-treated rams (Abecia *et al.*, 2017 and 2018).

Rams exposed to females in estrus in the breeding and non-breeding seasons exhibit a rapid increase in plasma LH and testosterone concentrations ('the female effect') (Schanbacher *et al.*, 1987; Gonzalez *et al.*, 1988), and close contact with estrous ewes increases the libido of the rams (Rodríguez Iglesias *et al.*, 1991). In goats, sexual behavior in the sexual rest was higher in males that had been in contact with females in estrus than it was in those that had been in contact with females in anestrus (Ramírez *et al.*, 2017); however, full contact and sexual interactions with estrus-induced goats failed to prevent seasonality in the LH and androgen plasma concentrations of bucks, even though bucks responded to the introduction of females by exhibiting acute increases in plasma LH and androgen (Ramírez *et al.*, 2019).

Ultrasonographic examinations of testicles are a potentially useful tool for predicting the reproductive performance of rams, although assessments of medium-term testicular variations in this species based on these tools are limited (Batissaco *et al.*, 2013; Samir *et al.*, 2018; Hedia *et al.*, 2019; Carvajal-Serna *et al.*, 2019). Color Doppler scanning has been used extensively to study testicular blood flow perfusion in humans and animals (Hedia *et al.*, 2019) based on the Resistive Index (**RI**), which is a measure of blood flow that reflects the resistance to blood flow caused by microvascular beds distal to the site of measurement, and the Pulsatility Index (**PI**), which quantifies pulsatility in the oscillations of the waveform. Changes in the color intensity of the pixels indicate whether there have been changes in the amount of testicular fluid (darker pictures indicate greater amounts of testicular fluid) (Ungerfeld and Fila, 2011). In horses, fertile and subfertile stallions differed significantly in all of the Doppler parameters measured, and fertile stallions had high vascular perfusion in the testicular artery (high testicular blood pulse), and subfertile stallions tended to have high PI and RI (high vascular resistance) (Ortiz-Rodríguez *et al.*, 2017). In rams, Ecotext 2 (white pixels) and blood flow measurements were negatively correlated with several sperm variables in semen samples collected 4 and 8 weeks after testicular evaluation (Carvajal-Serna *et al.*, 2019). The proportion of the sperms that were defective was positively correlated with RI and PI (Batissaco *et al.*, 2013), and the pampiniform plexus score was positively correlated with the number of colored pixels and negatively correlated with RI and PI.

Given that (1) continuous exposure to sexually activated rams or bucks induced by artificial photoperiod extends the ovarian activity of ewes and does in spring, which increases estrous expression (Abecia *et al.*, 2015; Delgadillo *et al.*, 2015), and (2) the continuous presence of sexually active rams and bucks prevents a reduction in plasma LH concentrations in ovariectomized-estradiol implanted (**OVX + E2**) ewes and goats in the seasonal anestrus (Muñoz *et al.*, 2017; Abecia *et al.*, 2019b), this experiment was designed to confirm whether these mechanisms can operate in the other direction, that is, from the ewe to the ram. We hypothesized that continuous exposure to ewes in estrus would inhibit the seasonal sexual rest in rams in spring. Plasma concentrations of LH and testosterone, sexual behavior and testicular changes were measured in rams that had been kept in continuous contact with ewes in estrus and rams that had been isolated from ewes.

Material and methods

Animals

On 1 March, 20 Rasa Aragonesa rams (mean (±SD) age = 16.60 ± 0.25 months; mean live weight (**LW**) = 70 ± 6 kg) were assigned to one of the two groups: the isolated group (**ISO**; $n = 10$), which was housed in a closed pen (5 × 7 m; 3.5 m^2/ram) and isolated entirely from other sheep, and the stimulated group (**STI**; $n = 10$), which was housed in a similar pen, but was adjacent to another pen that housed 12 ewes. Rams and ewes were separated by an openwork metal barrier, only, which allowed visual, olfactory and nose-to-nose contact between the sexes. Two weeks before the start of the experiment, and each week thereafter, four ewes were induced into estrus by intravaginal sponges impregnated with fluorogestone acetate (Sincropart, CEVA Salud Animal, Barcelona, Spain) for 14 days, which caused ewes to be sexually receptive for at least three consecutive days.

The two groups of rams were housed in barns that were separated by at least 300 m and were fed a diet that was formulated to fulfill their maintenance requirements, viz., 0.75 kg of pellets and 1 kg of barley straw per day, which provided 8.5 MJ of metabolizable energy per ram. The pellets contained barley (85%) and soy bean (15%). Ewes were fed

0.42 kg of pellets and 0.70 kg of barley straw per day, which provided 7.8 MJ of metabolizable energy per ewe. The pellets were the same than those offered to rams. Ewes and rams had unlimited access to water and a mineral supplement.

Measurements

To measure plasma testosterone levels, blood samples (March–May) were collected weekly from rams. In the first (4 March) and last (24 May) week of the experiment, LW, scrotal circumference (**SC**) and testicular width (**TW**) and length (**TL**) were recorded. Testicular volume (**TV**) was calculated as $0.0396 \times (\text{average TL}) \times (\text{SC})^2$ (El-Zelaky *et al.*, 2011).

On those days, a portable ultrasound scanner (7.5-MHz transducer; EXAGO, France) was used to quantify testicular structure by ultrasonography, as described by Abecia *et al.* (2019a). Briefly, three 124-frame videos of each testicle were recorded, positioning the probe transversely to the major axis of the testicle. The following image parameters were calculated (Ecotext software, Humeco, Spain): number of black, white or gray pixels (ECOTEXT 1, Ec1; ECOTEXT 2, Ec2; ECOTEXT 3, Ec3, respectively), density of tubules/cm^2, proportion (%) of the total area that was occupied by the lumen of the tubules in the parenchyma and mean diameter (μm) of the lumen of the seminiferous tubules. Thereafter, a color Doppler flow scan was performed in which the extent and direction of blood flow in the vessels of the testicles are indicated by color signals (red or blue at a 60° angle on the long axis of the vessel), and the color Doppler was used to identify signals of blood flow in the vessels of the pampiniform plexus. To identify the testicular artery and vein by Doppler analysis, an artery, for example, will have a waveform on the spectral graph that reflects the arterial pulse in each cardiac cycle (systole and diastole), but the flow in a vein is almost constant, that is, without a pulse. After the spectral pattern of the testicular artery is generated, the software calculates the RI (maximum velocity − minimum velocity/maximum velocity) and the PI (maximum velocity − minimum velocity/mean velocity), which indicates the pulsatility in the oscillations of the waveform (Batissaco *et al.*, 2013). Two measurements were taken for each variable in different locations along the path of the testicular artery. At least five waveforms were recorded per measurement, and a mean was calculated by the software. All ultrasonography measurements were performed by the same person. The Doppler gate was kept constant at 1 mm, and the high pass filter was set at 50 Hz.

In the last week of the experiment (27 May), five rams from each group were randomly selected and blood samples were collected by jugular catheters for 6 h at 20-min intervals, starting at 0800 h, to measure plasma LH concentrations. Luteinising hormone pulses were identified by the DynPeak algorithm (Vidal *et al.*, 2012).

Serving-capacity tests

At the end of the experiment (29 May), sexual activity of the rams was recorded by individual serving-capacity tests, as detailed by Abecia *et al.* (2019a), based on the previous test reported by Kilgour and Whale (1980) and Damián *et al.* (2015). Firstly, males were housed with three non-synchronized ewes for 20 min in a 15-m^2 pen, and the number of approaches to the ewes was recorded by one of four observers. Secondly, they were housed for 20 min in the same pen, with three ewes which were previously synchronized into estrus by intravaginal pessaries (Syncro-Part, CEVA Salud Animal, Spain) for 14 days, and the number of acts of flehmen, anogenital sniffing, approaches, attempted mounting and mounting were recorded by one of four observers. The definitions of sexual behaviors followed Calderón-Leyva *et al.* (2018) (flehmen: elevating the head and upper lip to taste and odor ewe urine or ambient odor; anogenital sniffing: sniff in the genital region of female; approaches: rubbing, licking or superficially nibbling the flank of the ewe with intensity; attempted mounting: stands behind the ewe and moves trying to copulate and mounting: intrusion of the penis into vagina of ewe with one or more thrusts and so, ejaculation can occur, with a backward elevation of the ram's head).

Hormonal assays

Heparinized 5-ml tubes were used to collect blood samples by jugular venipuncture; samples were immediately centrifuged at 3000×**g** for 20 min, and plasma was stored at −20°C. Luteinising hormone was assayed by radioimmunoassay (**RIA**) following Faure *et al.* (2005). The sensitivity of the LH assay was 0.1 ng/ml, and the intra-assay CV was 5.5%. All samples were run in a single assay. Plasma testosterone concentrations were measured by RIA following Hochereau-de Reviers *et al.* (1990). Sensitivity was 0.3 ng/ml. Samples were run in a single assay (intra-assay CV = 6%).

Statistical analysis

Paired *t* test for related samples was used to assess the statistical significance of differences between March and May values of LW, SC, testicular dimensions and those measurements derived from the testicular ultrasonography and color Doppler flow scanning. Differences between groups in mean plasma testosterone and LH concentrations, LH peak amplitude, the area under the curve, the onset of LH surge and its duration, and the behavioral test were assessed by ANOVA, with the presence of estrous ewes or isolation as the main effect. Differences in plasma LH concentrations of each group before and after ram introduction were compared by paired *t* test for related samples. Data are expressed as the mean ± SEM, and differences were considered significant at $P \leq 0.05$.

Results

Live weight and testicular measurements

In both ram groups, LW did not differ significantly between the start and the end of the experiment (Table 1). SC did not differ significantly between groups, although the SC of STI

Table 1 *Mean (±SEM) live weight, scrotal circumference, testicular width, length and volume, and image characteristics from testicular ultrasonography and color Doppler flow scanning of Rasa Aragonesa rams that had been housed in isolation of ewes or stimulated by ewes in estrus from March to May*

	Isolated		Stimulated	
	March	May	March	May
Live weight (kg)	71.0 ± 2.4	71.9 ± 2.0	69.0 ± 1.7	68.9 ± 1.8
Scrotal circumference (cm)	31.3 ± 0.6	31.7 ± 0.8	32.0 ± 0.8^{a}	33.0 ± 0.6^{b}
Testicular width (cm)	6.1 ± 0.2^{a}	6.57 ± 0.2^{b}	6.1 ± 0.2^{a}	6.5 ± 0.1^{b}
Testicular length (cm)	8.9 ± 0.3	9.3 ± 0.3	8.6 ± 0.3^{a}	9.5 ± 0.2^{b}
Testicular volume (cm^3)	350.4 ± 23.5	372.9 ± 25.8	354.1 ± 24.2^{a}	391.3 ± 17.7^{b}
Ecotext 1 (black pixels)	17.1 ± 4.6	25.2 ± 9.0	43.00 ± 17.7	16.9 ± 3.9
Ecotext 2 (white pixels)	94.3 ± 21.2^{a}	348.0 ± 73.7^{bx}	173.8 ± 48.6	160.2 ± 33.8^{y}
Ecotext 3 (grey pixels)	99.5 ± 1.5^{a}	107.0 ± 3.1^{b}	99.2 ± 2.3	102.6 ± 1.7
Tubular area (%)	7.9 ± 0.6	6.9 ± 0.6	9.1 ± 0.8^{a}	7.7 ± 0.5^{b}
Tubular diameter (μm)	102.8 ± 2.9^{a}	95.6 ± 3.7^{b}	112.1 ± 4.1^{a}	100.3 ± 2.3^{b}
Tubular density (tubules/cm^2)	142.4 ± 4.3	139.9 ± 3.7	137.6 ± 4.2^{a}	146.5 ± 4.4^{b}
Testicular blood pulse (bpm)	114.6 ± 4.2	107.7 ± 9.9	103.0 ± 2.4^{a}	123.2 ± 5.7^{b}
Pulsatility index	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.2^{a}	0.7 ± 0.1^{b}
Resistive index	0.5 ± 0.1	0.4 ± 0.1^{x}	0.6 ± 0.1	0.6 ± 0.1^{y}

^{a,b}Indicate significant differences between groups at $P < 0.05$.
^{x,y}Indicate significant differences between months at $P < 0.05$.

rams increased significantly ($P < 0.05$) between the start and the end of the experiment. In both groups, mean TW increased significantly ($P < 0.05$) between March and May and, in the STI group, only TL increased significantly ($P < 0.05$). Testicular volume did not differ significantly between the two groups, but STI rams had significantly ($P < 0.05$) higher TV in May than in March (Table 1).

Testicular scans

The number of black pixels in the testicular scans did not differ between treatment groups or months; however, in the ISO group, the number of white pixels was significantly ($P < 0.01$) higher in May than it was in March and significantly ($P < 0.01$) higher than it was in the STI group in May. In ISO rams, only the number of grey pixels increased significantly ($P < 0.05$) between March and May. In the STI group, tubular area (%) decreased significantly ($P < 0.05$) between March and May. In both groups, tubular diameter (μm) was significantly ($P < 0.05$) higher in March than it was in May. In the STI rams, density (tubules/cm^2) increased significantly ($P < 0.05$) between March and May (Table 1).

The color Doppler flow scanning revealed that, in the STI group, testicular blood pulse was significantly ($P < 0.01$) higher and PI was lower ($P < 0.05$) in May than it was in March. In May, the RI differed significantly between groups, so that STI rams had a significantly ($P < 0.05$) higher RI than did ISO rams (Table 1).

Testosterone and LH concentrations

The evolution of plasma testosterone concentrations throughout the studied period is presented in Figure 1. The two groups had similar plasma testosterone concentrations until the second half of April, when STI rams started to present significantly ($P < 0.05$) higher concentrations than did the ISO rams (Figure 1). STI rams had higher ($P < 0.01$) testosterone concentrations in May (6.64 ± 0.6 ng/ml) than did ISO rams (4.0 ± 0.6 ng/ml).

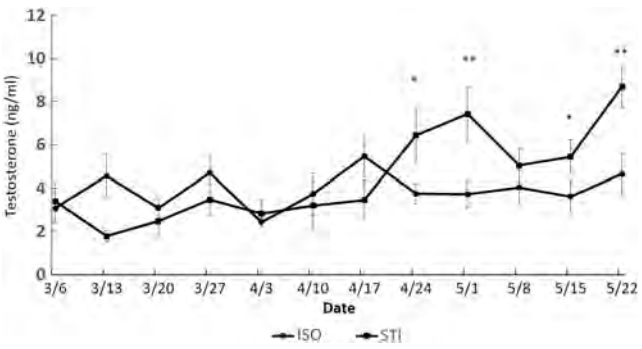

Figure 1 Mean (±SEM) weekly plasma testosterone concentrations (ng/ml) in Rasa Aragonesa rams that had been either housed in isolation of ewes (ISO) or stimulated (STI) by ewes in estrus from March to May (*$P < 0.05$; **$P < 0.01$).

The STI and ISO groups did not differ significantly in mean LH plasma concentrations (0.8 ± 0.5 *v.* 0.9 ± 0.4 ng/ml), number of LH pulses (2.1 ± 0.5 *v.* 2.2 ± 0.2) or pulse amplitude (2.0 ± 0.4 *v.* 3.2 ± 0.7 ng/ml, respectively) (Figure 2).

Serving-capacity tests

In the serving-capacity test with non-synchronized females, the groups did not differ significantly in mean number of approaches to ewes (ISO: 15.6 ± 2.53; STI: 13.2 ± 4.83); however, when rams were exposed to ewes in estrus, STI rams engaged in significantly ($P < 0.05$) more mountings (with intromission) than did ISO rams (Table 2). The groups did not differ significantly in the occurrences of flehmen, anogenital sniffing, approaches or mounting attempts.

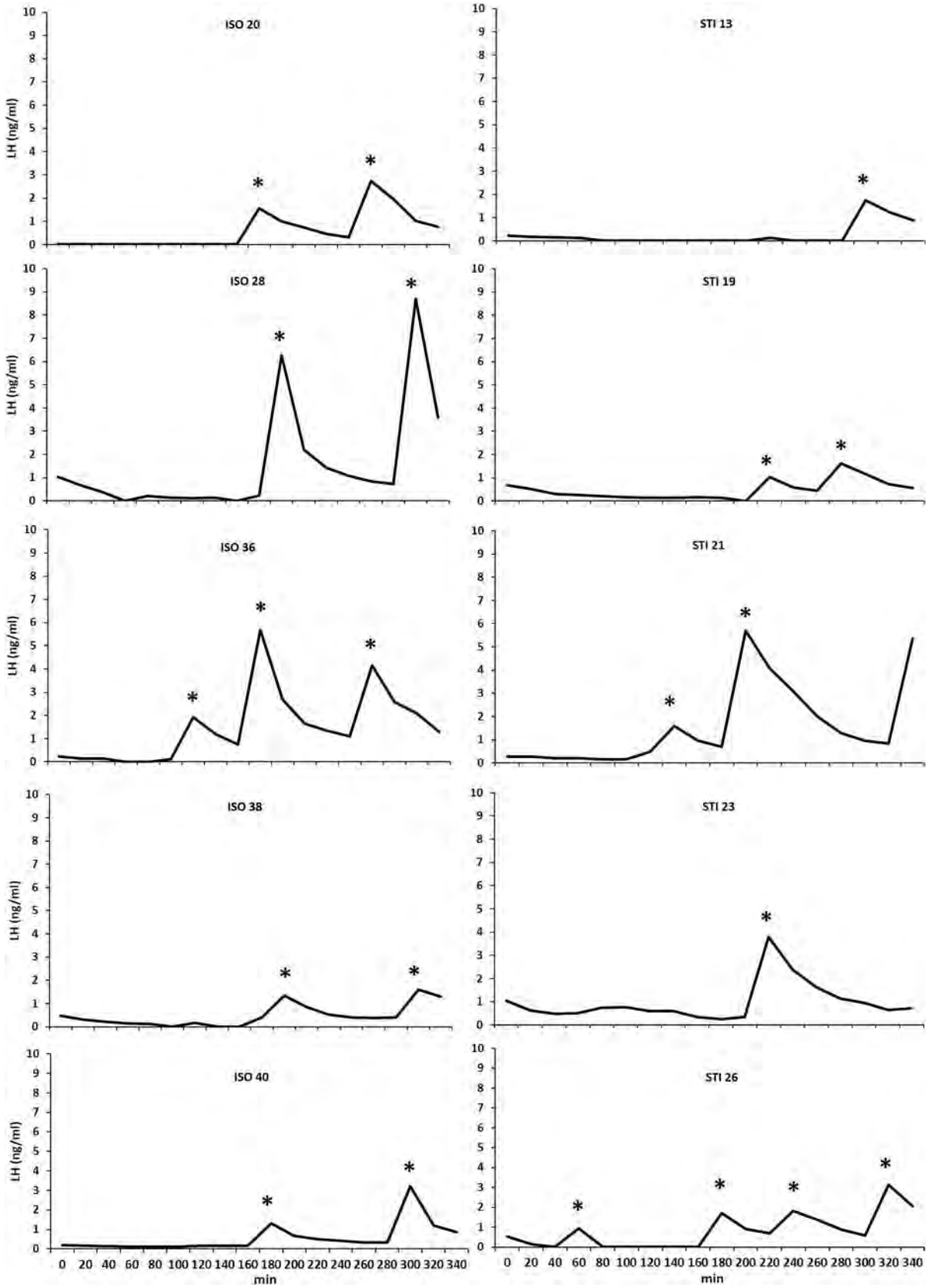

Figure 2 Plasma LH concentrations (ng/ml) of Individual Rasa Aragonesa rams that had been housed in isolation of ewes (ISO) or stimulated (STI) by ewes in estrus from March to May (* indicates an LH pulse).

Table 2 *Mean (±SEM) number of flehmen, anogenital sniffing, approaches, mounting attempts and mountings in 20 min in an individual (1 ram with three estrous ewes) serving-capacity test in May by Rasa Aragonesa rams that had been either housed in isolation (ISO) from ewes or stimulated (STI) by ewes in estrus from March to May*

	Flehmen	Anogenital sniffing	Approaches	Mounting attempts	Serves	Total
ISO	0.3 ± 0.3	9.8 ± 2.8	11.5 ± 3.8	1.5 ± 0.9	1.5 ± 0.5[a]	24.5 ± 4.7
STI	2.3 ± 1.1	16.5 ± 6.1	11.8 ± 4.2	0.8 ± 0.3	3.0 ± 0.4[b]	34.3 ± 8.7

[a,b]Indicate significant differences at $P < 0.05$.

Discussion

In this study, in the sexual rest period, only those rams kept in the presence of ewes in estrus exhibited an increase in SC and TV and their testosterone concentrations were higher than those of isolated rams after 2 months of exposure to ewes; however, this was not accompanied by an increase in LH secretion or a significant difference in sexual behavior, except STI rams exhibited significantly more intromissions than did ISO rams. In similar studies of goats, the presence of does in estrus stimulated the testicular activity of bucks, which produced higher quality semen and exhibited a larger increase in testicular fluid than did bucks that had been isolated from females, although the presence of does in estrus did not alter the typical seasonal pattern (Giriboni *et al.*, 2017), and full contact and sexual interactions with estrus-induced goats did not prevent seasonality in the LH and androgen plasma concentrations of bucks (Ramírez *et al.*, 2019).

Ultrasonography and the color Doppler flow scans indicated that the presence of estrous ewes led to better indicators of fertility and testicular tissue, especially at the time of deep anestrus (May), when exposed rams had significantly fewer white pixels, higher testicular blood pulse, a significant reduction in PI between March and May and a significant increase in tubular density than did isolated rams. All of those values have been shown to be either positively or negatively correlated with semen and testicular tissue quality (Batissaco *et al.*, 2013; Ortiz-Rodríguez *et al.*, 2017; Carvajal-Serna *et al.*, 2019).

In our study, the presence of ewes in estrus did not affect significantly LH pulsatility or plasma concentrations of LH and testosterone at the end of the experimental period. González *et al.* (1991a) reported that rams that had been stimulated by sexually receptive ewes exhibited a higher increase in LH pulse frequency and mean testosterone levels than did isolated rams. Similarly, Ungerfeld and Silva (2004) reported that rams that had been in continuous contact with estrual ewes exhibited a rapid increase in LH and testosterone levels in the first 4 days. Probably, the full contact between the sexes that was permitted in those experiments precipitated that immediate response because, in our experiment, rams and ewes were separated by a fence. González *et al.* (1991b) reported that, in sexually experienced rams, olfactory exposure to the urine, wool or vaginal secretions from sexually receptive females placed in a mask did not induce an endocrine response as measured by LH secretion pattern and testosterone concentrations. Furthermore, stimulation by the presence of sexually receptive females increased LH pulse frequency and testosterone concentrations, and hormonal parameter did not change in unstimulated control rams. Another experiment, which compared the LH secretion in ewes that had been exposed to the ram by visual and tactile cues (with fences), only, or with full physical contact between the sexes (with no fences), provided additional evidence of the importance of full physical contact between the sexes (Abecia *et al.*, 2002). In that study, we observed an absence of an initial LH response by ewes that had been separated from rams, until they re-joined a communal group, unlike the immediate response exhibited by ewes that had been housed in full contact with the rams. In addition, when full contact with vasectomized, sexually active rams was permitted, a reduction in plasma LH concentrations in OVX + E2 ewes in the seasonal anestrus was prevented (Abecia *et al.*, 2019a). However, we have also reported that individual confinement of sheep allowing visual, auditory, olfactory and tactile contact with their neighboring animals is not an obstacle for investigating particular hormonal interrelationships with multiple sampling procedures, as in the present experiment (María *et al.*, 2015).

In our study, the high testosterone concentrations in stimulated rams from April to the end of the experiment, and the increment of TV from March to May in this group of rams, might reflect an earlier recrudescence of the spermatogenic activity that is typically observed at the onset of the breeding season in these rams caused by the presence of ewes in estrus. At the beginning of the breeding season, an increase in testosterone concentration is necessary for the initiation, but not the maintenance, of spermatogenesis and normal mating activity (D'Occhio and Brooks, 1982). The higher T concentration presented by the STI rams in May might have been responsible, at least in part, for the high number of mating activities exhibited by these rams in the serving-capacity test, although STO and ISO rams did not differ significantly in the other behaviors.

In conclusion, after 3 months in the continuous presence of ewes in estrus in spring, rams increased their TV and some testicular echogenic characteristics were modified compared to isolated rams. Although exposed rams also had higher levels of testosterone after 2 months in the presence of estrous ewes, their LH pulsatility at the end of the study was not modified.

Acknowledgements

The work was supported by Ministerio de Ciencia y Tecnología (Spain), grant number AGL2017-82118-R. We thank José Antonio Ruiz and Antonio Barrio for their help in the care of the animals, and Bruce MacWhirter for the English revision of the manuscript.

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Declaration of interest

The authors declare that there are no conflicts of interest.

Ethics statement

The experiment was conducted at the experimental farm of the University of Zaragoza, Spain (41°40′ N 0°53′ W). The Ethics Committee for Animal Experiments at the University of Zaragoza approved all of the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Software and data repository resources

None of the data was deposited in an official repository.

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