



Presence of photoperiod-melatonin-induced, sexually-activated rams in spring advances puberty in autumn-born ewe lambs



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ABSTRACT

The objective of this study was to determine the effect of sexually activated (by specific photoperiodic treatments) vasectomized rams on the onset of puberty in autumn-born ewe lambs in spring. Photoperiodic-treated rams were assigned to one of three groups, which were sequentially exposed to two months of long days (16 h light/d) and given three melatonin implants at the end of the long days (sexually-activated-rams; SAR): SAR1 ($n = 5$; 1 December–31 January), SAR2 ($n = 5$; 1 Jan–28 February), and SAR3 ($n = 5$; 1 February–31 March). Control rams (CR; $n = 4$) were exposed to the natural photoperiod. On 1 March, 50 ewe lambs born in September were assigned to the SAR rams (SAR-treated; $n = 25$) or the CR rams (CR-treated; $n = 25$). SAR-treated ewe lambs were housed with SAR1 rams from 1 March. SAR2 rams replaced SAR1 rams (14 April), which were replaced by SAR3 rams (22 May) until 30 June. CR-treated ewe lambs were housed with the unstimulated rams. Ovulation was identified by weekly plasma progesterone concentrations and estrous behavior, as indicated by colored rumps. A greater proportion of SAR than CR ewe lambs ovulated in April (52% vs. 0%) and May (68% vs. 0%) ($P < 0.0001$), and were in estrus in May (64% vs. 0%) and June (92% vs. 24%) ($P < 0.0001$). The presence of SAR rams reduced ($P < 0.0001$) mean (\pm SD) age (d) at first ovulation and estrus (235 ± 28 [7 May] and 257 ± 24 [29 May], respectively), compared to that of CR-treated ewe lambs (277 ± 5 [18 June] and 302 ± 16 [14 July], respectively). In conclusion, the presence of photoperiod-melatonin-induced, sexually activated rams in spring, advanced puberty in autumn-born ewe lambs. Using this technique might provide an effective and sustainable means of increasing the productive life of ewes, while avoiding the use of hormonal treatments.

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1. Introduction

Reducing the unproductive periods in a breeding ewe lamb increases the total lamb crop production in lifetime. The period from weaning until first breeding is one of the most unproductive (Gordon, 1997). The advantages of breeding at an early age include a reduction in the

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maintenance costs of the replacement flock, a shortened generation interval, the synchronization of lambing with adult ewes, and an increase in lifetime production. The onset of puberty in ewe lambs is multifactorial in nature, of which month of birth influences the age of puberty. Ewe lambs born in winter or spring generally reach puberty at a younger age than those born during autumn, reaching a later stage by 10–12 months (Valasi et al., 2009). Apparently, the delay in puberty in autumn-born ewe lambs arises from a prolonged hypersensitivity to the estradiol negative-feedback (Foster, 1994). In spring-born females, the reduced response to estradiol feedback inhibition of LH secretion diminishes in spring-born females at 26–30 weeks of age, during a period of decreasing day length; however, autumn-born lambs remain hypersensitive to estradiol at that age because of increasing day length.

Exposure to two months of long days (16 h light/day) has been shown to be very efficient in sexually-activating rams, advancing puberty in young rams, causing a substantial increase in sperm production in adult rams, which allows earlier use of these animals in progeny tests for artificial insemination, and can cause a significant increase in testicular weight (Chemineau et al., 1992). Recently, we demonstrated that the year-round presence of sexually active bucks, stimulated by this particular extra-light treatment during autumn and winter, can prevent seasonal anestrus in goats (Delgadillo et al., 2015). In sheep, continuous exposure in Mediterranean ewes to sexually-activated rams that had been stimulated by artificial photoperiod and melatonin implants, prolonged ovarian activity in spring and increased estrous expression (Abecia et al., 2015). Furthermore, the introduction at weaning of sexually-activated rams advanced the resumption of estrous activity in adult ewes in spring (Abecia et al., 2016). Thus, the capacity of sexually active males to override the photoperiodic control of female sexual activity has provided a new and sustainable approach to the reproductive control of small-ruminant flocks, in which photoperiod is the main factor influencing the seasonality of reproduction.

Socio-sexual signals between the sexes can also influence the onset of puberty in sheep, so that ewe lambs continuously exposed to vasectomized rams advanced sexual maturation (Kassem et al., 1989; Kenyon et al., 2005). Breeding ewe lambs for the first time at eight months of age is a potential means of increasing lifetime reproductive performance (Kenyon et al., 2012). However, this is difficult to achieve in autumn-born ewes, probably because they have not reached puberty. Since it has been demonstrated that light-treated rams maintain reproductive activity in anestrus females, and that the exposure of males to young females induce puberty, we tested the hypothesis that the presence of sexually activated (by specific photoperiodic treatments) vasectomized rams can induce the onset of puberty in autumn-born ewe lambs in spring.

2. Material and methods

2.1. Experimental design and groups

The study included 19 vasectomized, sexually experienced adult Rasa Aragonesa rams (5–8 years of age),

which had an average live weight (LW) of 105 ± 4 kg (mean \pm S.E.M.) and a mean body condition score (BCS; on a scale of 0–5, where 0 = emaciated and 5 = obese; Russel et al., 1969) of 3.25 ± 0.07 . Control rams (CR; $n=4$) were kept in a shaded, open pen and exposed to natural photoperiod (15 h and 12 min, and 9 h and 10 min of light at the summer and winter solstices, respectively). Photoperiodic-treated rams ($n=15$) were induced into a sexually-active (SA) state by exposure to artificial long days followed by the application of subcutaneous melatonin implants (Melovine, CEVA Salud Animal, Barcelona, Spain). Males were kept permanently in a shaded, open pen under natural photoperiod before the photoperiodic treatments, and assigned to one of three groups. One group was exposed to two months of long days (16 h of light/d) in a pen (5 m x 7 m; 8.75 m²/ram) between 1 December and 31 January (SAR1 group, $n=5$), the second group was exposed to long days between 1 January and 28 February (SAR2 group, $n=5$), and the third group was exposed to long days between 1 February and 31 March (SAR3 group, $n=5$). Artificial light was provided in the morning (06:00–09:00) and the evening (16:00–22:00), which was controlled by an electronic timer, and light intensity was ≥ 300 lx at the eye-level of the animals (Chemineau et al., 1992).

At the end of the long-day period, rams were returned to natural photoperiod conditions and each given three subcutaneous melatonin implants (SAR1: 1 February; SAR2: 1 March; SAR3: 1 April). Rams become insensitive or refractory to melatonin after about 16 weeks of exposure (Lincoln and Clarke, 1997); therefore, to ensure that ewe lambs had continuous exposure to sexually-activated rams, three groups of photoperiodic-treated rams were used in the experiment.

Fifty Rasa Aragonesa ewe lambs born in September (15 September \pm 10 days), were assigned to one of two groups, which were balanced for LW and BCS, and allocated to different shaded, open pens on 1 March. The sexually-activated-ram group (SAR-treated; $n=25$) was housed with two photoperiodic-stimulated SAR1 rams from 1 March. SAR2 rams replaced SAR1 rams on 14 April, which were replaced by SAR3 rams on 22 May and remained with the ewes until 30 June. The control-ram group (CR-treated; $n=25$) was housed with the unstimulated CR rams throughout the experiment (1 March–30 June). Both SAR and CR rams were managed in the same manner, such that ewe lambs were housed with two males, which were rotated every two weeks with other two rams in the same group. To prevent a 'novel male' effect on ewe lambs when the treated rams in the same group were rotated, or when SAR2/SAR3 rams replaced SAR1/SAR2 rams, they were housed in pens adjacent to those of the females, and rams were separated from ewes by an openwork metal barrier only, which allowed visual, olfactory, and nose-to-nose contact between the sexes. The two groups of ewes were housed in different barns that were separated by at least 300 m.

To estimate age at puberty in those ewe lambs that were not detected in estrus based on the behavior of vasectomized rams, four Rasa Aragonesa rams were introduced on 1 July and remained with ewe lambs until 31 August (breeding season; Forcada et al., 1992). To prevent a

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