Theriogenology 84 (2015) 1549–1555

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Continuous exposure to sexually active rams extends estrous activity in ewes in spring

J.A. Abecia [a,*], P. Chemineau [b], J.A. Flores [c], M. Keller [b], G. Duarte [c], F. Forcada [a], J.A. Delgadillo [c]

[a] Departamento de Producción Animal y Ciencia de los Alimentos, IUCA, Universidad de Zaragoza, Zaragoza, Spain
[b] Physiologie de la Reproduction et des Comportements, INRA, CNRS, Université de Tours, IFCE, Agreenium, Nouzilly, France
[c] Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, Mexico

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 1 August 2015
Accepted 4 August 2015

Keywords:
Ram
Ewe
Ovulation
Estrus
Season

ABSTRACT

Sexual activity in sheep is under photoperiodic control, which is the main environmental factor responsible for the seasonality of reproduction. However, other natural environmental factors such as presence of conspecifics can slightly influence the timing of onset and offset of the breeding season. In goats, we have found that the continuous presence of bucks that were rendered sexually active out of season by previous exposure to long days, prevented goats from displaying seasonal anestrus, which suggests that the relative contribution of photoperiod in controlling seasonal anestrus should be reevaluated in small ruminant species. The aim of this study was to assess whether the presence of sexually active rams that had been stimulated by artificial photoperiod and melatonin implants, reduces seasonal anestrus in sheep, by prolonging ovulatory activity in spring. Ewes were assigned to one of two groups ($n = 16$ and 15), which were housed in two separate barns, and kept in contact, either with the treated or the control rams between March and July. Vasectomized rams were either exposed to 2 months of long days followed by the insertion of three subcutaneous melatonin implants (treated rams, $n = 8$), or exposed to natural light conditions (control rams, $n = 2$). Estrus was monitored daily, and weekly plasma progesterone analyses indicated ovulatory activity. Ewes that were exposed to treated rams exhibited a higher proportion of monthly estrus than ewes exposed to the control rams ($P < 0.05$). Thirteen of 15 ewes (one ewe was not considered because of the presence of persistent CL) exposed to stimulated rams exhibited estrous behavior in a cyclic manner. In contrast, all ewes exposed to control rams stopped estrous activity for a period of time during the study, such that this group exhibited a significantly longer anestrous season (mean ± standard error of the mean 89 ± 9 days) than did the ewes housed with treated rams (26 ± 10 days; $P < 0.0001$). Among 15 ewes housed with treated rams, 13 of them exhibited continuous ovulatory activity between March and July, whereas one stopped in June and two in July. All ewes kept with control rams stopped ovulating for some time; consequently, those ewes had a longer anovulation period than did the group exposed to treated rams (3 ± 3 vs. 18 ± 7 days, respectively; $P < 0.05$). In conclusion, continuous exposure to sexually activated rams induced by artificial photoperiod and melatonin implants in spring extended the ovarian activity of ewes in spring, which results in an increase in estrous expression.

Preliminary results of this experiment have been presented in the XVI *Jornadas de Producción Animal*, Zaragoza, Spain, 19-20 May 2015.

* Corresponding author. Tel.: +34 876554159; fax: +34 976761590.
E-mail address: alf@unizar.es (J.A. Abecia).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.08.002

1. Introduction

Seasonality in reproduction ensures that births occur at the optimal time of the year, usually at the end of winter and/or the beginning of spring, which allows the newborn to survive and grow under favorable conditions of temperature and food availability [1]. Sexual activity in sheep is under photoperiodic control, which is the main environmental factor responsible for the seasonality of reproduction; however, other natural environmental factors such as nutrition, temperature, or presence of conspecifics can slightly influence timing of the onset and offset of seasonal reproduction [2–4]. When rams are housed with anovulatory ewes that have been previously isolated from rams, a proportion of the flock will ovulate, display estrus, and become pregnant [5]. This effect has been called the "ram effect".

In addition, rams exhibit a seasonal decrease in sexual behavior and spermatogenesis at about the same time that ewes are in sexual rest, but with a 1 to 2-month advance in phase [1,6]. Melatonin implants advance testicular growth and improve sperm production, although a light treatment involving long days is required before the insertion of implants to stimulate testicular growth, increase sexual behavior, sperm production, and fertility [7].

Recently, we found that the year-round presence of sexually active bucks, stimulated by extra-light treatment during autumn and winter, can prevent seasonal anestrus in goats [8]. When these bucks were removed during the natural season of anovulation, females stopped cycling and entered anestrus, immediately. The capacity of the male goats to override the photoperiodic control of female sexual behavior has generated new questions for research in not only goats but also sheep, in which photoperiod is the main factor influencing the seasonality of reproduction [1,9].

The objective of this study was to determine whether the presence of rams that had been sexually stimulated by specific photoperiodic treatments can prolong the sexual activity of ewes throughout the seasonal anestrus.

2. Material and methods

2.1. Experimental design

The study included three groups of vasectomized, sexually experienced adult Rasa Aragonesa rams (5–8 years of age), which had a live weight (LW) of 93 ± 2 kg (mean ± standard error of the mean) and a mean body condition score (BCS; on a scale of 0–5, where 0 = emaciated and 5 = obese [10]) of 3.35 ± 0.07). Control rams (n = 2) were kept in a shaded, open pen (6 × 12 m) and exposed to the natural photoperiodic conditions (15 hours and 12 minutes, and 9 hours and 10 minutes of light, at the summer and winter solstices, respectively). Photoperiodic-treated rams (n = 8) had been rendered sexually active by a treatment consisting in long days followed by the application of subcutaneous melatonin implants (Melovine; CEVA Salud Animal, Barcelona, Spain). These implants release melatonin progressively and at sufficiently high plasma concentrations for a period of 70 to 90 days to simulate short days; they are positioned at the base of the ear and do not need to be removed as they are biodegradable. Males were permanently kept in a shaded, open pen (6 × 12 m) under natural photoperiodic conditions before the photoperiodic treatments, and assigned to one of two groups. One group was exposed to 2 months of long days (16 hours of light/day) in a closed pen (5 × 7 m; 8.75 m^2/ram), between 1 December and 31 January (T1 group, n = 4); the other group was exposed to long days between 1 January and 28 February (T2 group, n = 4). Lighting was controlled by an electronic timer, and light intensity was at least 300 lx at the level of the eyes of the animals. At the end of long days, rams were returned to natural photoperiod conditions and given three subcutaneous melatonin implants (T1: 1 February; T2: 1 March). These two groups of photoperiodic-treated rams were used in the experiment to ensure a continuous exposure of ewes to sexually activate rams throughout the whole experimental period, as rams become insensitive or refractory to about 16 weeks of exposure to melatonin [11].

Nonpregnant multiparous Rasa Aragonesa ewes, with an LW of 67 ± 2 kg and a BCS of 3.35 ± 0.07, were assigned to one of two groups, which were balanced for LW and BCS, and allocated to different shaded, open pens (8 × 4 m). One group (treated ram group; n = 16) was housed with two photoperiodic-stimulated T1 rams, which were rotated every 2 weeks with the other two T1 rams, from 27 February to 30 April. T2 rams replaced T1 rams on 1 May and remained with the ewes until 31 July; T2 rams were managed in the same way than T1 rams, so that ewes were housed with two T2 males rotated every 2 weeks with the other two T2 rams. The other group (control ram group; n = 15) were housed with the unstimulated rams.

To prevent a "novel male" effect on ewes when the treated rams of the same group were rotated, or when T2 rams substituted T1 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 separated by at least 300 m.

2.2. Measurements

From 27 February to 31 July, ovarian status was assessed once per week based on plasma progesterone concentrations (P4). Blood samples were collected by jugular venipuncture and placed into heparinized tubes. Samples were centrifuged at 3500 × g for 30 minutes, and the plasma fraction was stored at −20 °C until hormone concentrations were measured. Concentration of plasma P4 higher than 1 ng/mL of plasma is indicative of a previous ovulation. This threshold concentration, in nonpregnant females of the ruminant species, is indicative of the presence of at least one active CL, the only source of P4 in substantial amounts in the blood, and which is indicative of a recent ovulation [12–14]. To facilitate the daily monitoring of estrus, rams wore marking harnesses, which left colored marks on the rumps of the ewes. Therefore, estrous behavior was monitored daily by identifying the marks left by the harness.