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SHORT COMMUNICATION

Comparison of four strategies of ram management in a semen collection centre

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Abstract

Thirty Merino rams were used to determine the effect of four management systems of rams on semen quality during the anoestrous season. Animals were divided into four groups: Artificial-Photoperiod group (AP; $n = 8$), which were isolated from females and exposed to artificial long days (16 hr/d) from 1 Feb to 15 Mar; Natural-Photoperiod (NP; $n = 8$), isolated from females and exposed to the natural photoperiod throughout the experiment; Oestrous-Ewe group (EE; $n = 7$), housed in a pen adjacent to another pen that housed three ewes in oestrus, and Anestric-Ewe group (AE; $n = 7$), housed adjacent to another pen that housed three ovariectomized ewes. From 20 Mar to the end of May (10 weeks), semen samples were collected weekly, and blood samples were collected to determine plasma testosterone concentrations. Mean plasma testosterone concentrations, ejaculate volume and reaction time were not affected either by treatment or week. There was a significant effect ($p < .01$) of ram treatment on sperm concentration, and both TM y PM, and their interaction, were significantly affected by group and week ($p < .001$). Rams exposed to ewes in oestrus presented the largest sperm concentration ($p < .05$) compared with the other three groups, although they had the lowest total and progressive motilities ($p < .01$). In conclusion, management strategy in spring affects semen quality of rams, with the presence of ewes in oestrus being the best plan to increase sperm concentration.

KEYWORDS
artificial photoperiod, presence ewes, ram, semen

1 | INTRODUCTION

Reproduction in sheep is mainly driven by photoperiod, so that rams located in temperate latitudes present a reduction in testicular volume and diameter and an alteration of their hormonal profiles during the non-reproductive season (Lincoln et al., 1990). As a consequence, a decrease in quantitative and qualitative semen production has been observed (Colas, 1980), with a seasonal reduction in the number of seminal doses to inseminate in spring. Light treatments of rams have been used to counteract this phenomena, such as that reported by Chemineau et al. (1988), who observed that semen quality of light-treated Ile-de-France rams remained at the same level as in control animals during the natural breeding season. On the contrary, the continuous presence of oestrous ewes in the breeding and

non-breeding seasons produces a rapid increase in rams' plasma LH and testosterone concentrations and increases the libido of the rams (review: Abecia et al., 2020).

The aim of this work was to determine the effect of four management systems of rams (changes in the photoperiod or the presence of ewes) in a semen collection centre, on semen quality during the anoestrous season.

2 | MATERIAL AND METHODS

The study was conducted at the experimental farm of IFAPA Hinojosa del Duque, Spain (latitude: 38° 69' N, longitude: 5° 11' E). 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 (RD 53/2013), which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Thirty Merino rams were divided into four groups: Artificial-Photoperiod group (AP; $n = 8$), which were isolated from females and exposed to artificial long days (16 hr/d) from 1 Feb to 15 Mar, when they were changed to the natural photoperiod until the end of the experiment; Natural-Photoperiod (NP; $n = 8$), isolated from females and exposed to the natural photoperiod throughout the experiment; Oestrous-Ewe group (EE; $n = 7$), housed from 1 Feb in a pen adjacent to another pen that housed three ewes, which were induced into oestrus by weekly injections of beta-oestradiol (Sigma-Aldrich, Madrid, Spain), and Anestric-Ewe group (AE; $n = 7$), housed from 1 Feb in a pen adjacent to another pen that housed three ovariectomized ewes. Rams and ewes in groups EE and AE were separated by an openwork metal barrier which allowed visual, olfactory and nose-to-nose contact between the sexes. Lighting was controlled by an electronic timer in the AP group, using artificial light in the morning (06:00 to 09:00) and at night (16:00 to 22:00), and light intensity was at least 300 lx at the level of the eyes of the animals.

From 20 Mar to the end of May (10 weeks), semen samples were collected weekly in an artificial vagina at 35–40°C lubricated with petroleum jelly. Each collection day, a routine-simplified semen analysis was performed that included volume and concentration, and total motile (TM) sperm, and progressive (PM), sperm subpopulations measured in a computer-assisted sperm analysis (CASA). Reaction time of each ram (the interval between first contact with the teaser ewe and ejaculation) was recorded. Rams were previously trained to be semen collected. Blood samples were collected the same day to determine plasma testosterone concentrations. They were analysed by Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA), with an intra-assay variation of 8% and inter-assay variation of 7.5%.

Hormonal levels and semen quality parameters were evaluated statistically based on a multifactorial model that included group and week as fixed effects and used the least squares method of the GLM procedure in SPSS v.26 (IBM Corp. Released, 2019). Secondly, they were compared within fixed effects by an ANOVA. To assess the effect of ram management, a post hoc Fisher's least significant difference (LSD) test was performed.

3 | RESULTS

Both mean plasma testosterone concentrations and ejaculate volume and reaction time were not affected either by treatment or week (Table 1). There was a significant effect ($p < .01$) of ram treatment on sperm concentration, and both TM y PM, and their interaction, were significantly affected by group and week ($p < .001$). Rams exposed to ewes in oestrus presented the largest concentration ($p < .05$) compared with the other three groups (Table 1), although their sperm had the lowest TM and PM ($p < .01$). The best motility was observed in the AE group.

4 | DISCUSSION

If we consider that the group of rams exposed to the natural photoperiod and isolated from females is the usual management found in the semen collection centres, the continuous presence of ewes in oestrus was the only strategy that modified semen quality, increasing sperm concentration, although with a lower motility. We have reported that after three months in the continuous presence of ewes in oestrus in spring, rams had higher testicular volume than isolated rams, and some testicular echogenic parameters were modified (Abecia et al., 2020), so it is likely that this mechanism could be responsible for the higher sperm concentration observed in this experiment. Moreover, Ungerfeld and Silva (2004) reported an increase

TABLE 1 Mean (±S.E.) plasma testosterone concentrations (T, ng/ml), reaction time (RT, sec), and volume (ml), sperm concentration (spz/ml), total motility (TM, %) and progressive motility (PM, %) of rams either exposed to an artificial photoperiod (AP; $n = 8$) (16 hr/d) from 1 Feb to 15 Mar; to the natural photoperiod (NP; $n = 8$), to ewes in oestrus (EE; $n = 7$), or to anestric-ovariectomized ewes (AE; $n = 7$) (a,b; c,d indicate significant differences $p < .05$)

	T	RT	Volume	Concentration	TM	PM
AP	2.9 ± 0.3	81.5 ± 10.9	1.32 ± 0.06	$3.99x10^9$ ±1.74^a	68.3 ± 2.3^{ac}	38.8 ± 2.2^{ac}
NP	3.4 ± 0.2	72.7 ± 9.2	1.21 ± 0.04	$4.05\ x10^9$ ±1.22^a	70.3 ± 2.2^{ad}	48.2 ± 2.0^{ad}
EE	3.7 ± 0.3	74.7 ± 8.2	1.31 ± 0.05	$4.62\ x10^9$ ±1.20^b	60.1 ± 2.4^{ad}	30.9 ± 2.0^{ad}
AE	3.6 ± 0.3	54.4 ± 9.3	1.20 ± 0.04	$4.16\ x10^9$ ±1.47^a	76.2 ± 1.7^b	51.4 ± 1.9^b

in testicular firmness and resilience, which may be caused by an increase in testicular fluid content, in rams exposed to oestrual ewes. Rams in that study also increased their testosterone concentrations, although this fact was observed only at the end of the 3 months period of exposition to oestrous ewes.

On the contrary, light treatment of rams was not able to improve semen quality, in opposite to that previously demonstrated by Chemineau et al. (1988); it is likely that the artificial photoperiod was imposed late (Feb–Mar) for a not very seasonal breed as the Merino, which has a short anoestrous period (Gómez-Brunet et al., 2012), so that the difference between the light regime (16 hr) and the natural photoperiod throughout the experiment (12–14.5 hr) was not enough to induce neuroendocrine changes. In fact, that Spanish Mediterranean breeds of sheep, with their reduced seasonality, appear to use an endogenous circannual rhythm to generate their reproductive transitions (Gómez-Brunet et al., 2008).

In conclusion, management strategy in spring affects semen quality of rams, with the presence of ewes in oestrus being the best alternative to increase sperm concentration.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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