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Genome-wide association studies for reproductive seasonality traits in Rasa Aragonesa sheep breed

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ABSTRACT

Sheep breeds from Mediterranean area show reproductive seasonal patterns of oestrous behaviour and ovulatory activity, mainly regulated by variation in the photoperiod. Maximal reproductive activity is associated with short days from August to March. The aim of this study therefore was, to identify new SNPs and genes associated to reproductive seasonality in sheep by using the Illumina OvineSNP50 Beadchip. A total of 239 adult Rasa Aragonesa breed ewes from one flock were controlled from January to August. Three reproductive seasonality traits were considered: the total days of anoestrus (TDA), based on weekly individual plasma progesterone levels and defined as the sum of days in anoestrus, considering anoestrus those periods with three or more consecutive P4 concentrations lower than 0.5 ng/ml; the progesterone cycling months (P4CM), defined for each ewe as the rate of cycling months between January and August based on progesterone determinations and the oestrus cycling months (OCM), defined for each ewe as the rate of months cycling between January and August based on oestrus records. Genotyping of 123 ewes was performed with the OvineSNP50 Infinium Beadchip. After the quality control (QC) performed on the raw genotypes, a total of 47,206 SNPs distributed over the 27 ovine chromosomes and 110 ewes were included in subsequent analyses. Principal component analysis revealed a substructure within the total dataset and identified 4 principal clusters in the experimental flock. None of the SNPs overcame the genome-wide significance level ($P = 1.06 \times 10^{-6}$). However, the SNPs OAR4_66002395 (9.41E-6), and OAR8_25877010 (1.86E-5) reached the genome-wide suggestive significance level (set to 2.32×10^{-5}) for TDA and P4CM traits, respectively, while OAR23_14608581 was significant for both TDA (2.02E-5) and P4CM (1.05E-5) traits. Five SNPs evidenced association at chromosome-wise level: SNPs OAR4_66002395, OAR23_14608581 and s20800 (DTA), and OAR8_25877010, OAR23_14608581 and s48474 (P4CM). Several genes related to circadian and circannual rhythms were found close to these SNPs: *NPSR1* (SNP OAR4_66002395), *HS3ST5* (SNP OAR8_25877010), RPTOR (SNP s48474), and *NPTX1* (SNP s48474) and could be considered as candidate gene related to TDA and P4CM traits.

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1. Introduction

Seasonal patterns of oestrous behaviour and ovulation are important factors limiting the production efficiency of sheep livestock. Sheep breeds from Mediterranean area show reproductive seasonality, mainly regulated by variation in the photoperiod. Maximal reproductive activity is associated with short days, with the highest percentage of ewes exhibiting ovulatory activity from August to March. This reproductive seasonality induces great variation in lamb production and, therefore, in the market price of lamb meat. Hormonal treatments are widely used in some countries to control reproductive activity out of the breeding season, but the increasing demand for hormone-free products leads to search

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for alternative methods such as the male effect or the use of genetic markers. In this sense, only two genes affecting reproductive seasonality traits have been successfully identified, the *melatonin receptor subtype 1A* (*MTNR1A*) [1–11], and the *arylalkylamine N-acetyltransferase* (*AANAT*) [12]. Arylalkylamine N-acetyltransferase is involved in the biosynthesis of melatonin, and then controls daily changes in melatonin production. *MTNR1A* has been repeatedly proposed as candidate gene and seems to play a key role in the control of photoperiod-induced seasonality mediated by the circadian concentrations of melatonin [13–15]. However, Hernandez et al. [16] showed that *MTNR1A* genotype had no association with reproductive seasonality, suggesting that the effect of the *MTNR1A* polymorphisms may depend on the genetic background and/or of environmental conditions, thereby restricting its usefulness to that of a breed-specific genetic marker. Finally, a genetic mapping of quantitative trait loci (QTL) for aseasonal reproduction in sheep using microsatellites revealed seven chromosomes that could harbour putative QTLs for one or more phenotypes used to described this trait [17]. Functional genomic studies of genes associated with circadian and circannual rhythms have revealed additional candidate genes involved in seasonal breeding in sheep such as *ARNTL*, *CSNK1E*, *CLOCK*, *CRY1*, *PER1*, PER2, or *NPAS4* [18–20], but no association studies have been carried out with these genes.

In Rasa Aragonesa, as in other Mediterranean sheep breeds, the occurrence of oestrous behaviour and ovulation in spring depends on management and feeding conditions, so that the proportion of ewes ovulating spontaneously in flocks is around 25% with large individual differences [21]. This spring ovulatory activity is under genetic control with heritability and repeatability values of 0.20 and 0.30, respectively [22], but selection for improved fertility in accelerated lambing systems is particularly challenging because of the complexity of the system [23].

Sufficient dense SNPs arrays have recently been released in sheep to study genome-wide variants associated to traits of interest [24]. Genome-wide association studies (GWAS) have been applied to detect and localize candidate genes for quantitative traits that could expand marker-assisted selection in sheep [25–30]. Therefore, the main objective of this study is to identify new SNPs and genes associated to reproductive seasonality in sheep by using the Illumina OvineSNP50 Beadchip.

2. Materials and methods

2.1. Ethics statement

All experimental procedures were performed in accordance with the guidelines of the European Union (2003/65/CE) and Spanish regulations (RD 1201/2005, BOE 252/34367–91) for the use and care of animals in research. No hormonal treatments were applied to ewes during the study.

2.2. Animal resources

Phenotypic seasonality data were obtained from a Rasa Aragonesa sheep flock managed in an experimental farm (“Pardina de Ayés”) owned by Oviaragón S.C.L., located in the Pre-Pyrenees (Ayés, Sabiñánigo, Huesca; North-Eastern Spain, 42° 29′ 48.55” N 0° 23′ 37.54”, 790 m above sea level). The experimental period spaned from January to August 2012. The average annual rainfall is bimodally distributed, with peaks in spring and autumn, dry summers and some precipitation in the form of snow in autumn and winter. The flock was composed of 239 adult ewes in two age groups: young (all: 1.9 years, n = 84) and mature (5.2–7.2 years, n = 155; 5.5 ± 0.5; mean ± SD) at the beginning of the experiment. Individual liveweight (LW) and body condition score (BCS) on a 1 to 5 scale [31] were assessed every three weeks. Mean LW and BCS were similar in both age groups. Pooled overall means and standard deviations for the whole experimental period were 52.5 ± 7.7 kg and 2.9 ± 0.3 for LW and BCS, respectively. Ewes were not mated to fertile rams either during the experimental period or throughout the preceding year. Ewes that had lambed in the preceding year from matings carried out two years backwards were not considered for this study. Ewes were kept indoors and fed a commercial concentrate from October to March. From that month to the end of the experimental period (August) ewes were grazed on mountain pastures and received the same commercial concentrate *ad libitum*, according to the local traditional system. All ewes were handled in a single lot and weresubjected to the same management, nutrition and environmental conditions.

2.3. Measurement of reproductive seasonality traits

Three reproductive seasonality traits were considered. The first one was the total days of anoestrus (TDA) based on weekly plasma progesterone levels from January to August (35 samples per ewe). Progesterone was determined using a comercial ELISA kit designed for ovine plasma (Ridgeway Science, St. Briavels, Gloucestershire, UK) and the patterns of progesterone levels of ewes were plotted. A period of anoestrus was considered to have occurred when progesterone levels were under the threshold of 0.5 ng/ml in three or more consecutive samplings, while only two consecutive samplings under the threshold was considered an unfrequent occurrence compatible with normal cyclicity, according to Teyssier et al. [7]. In other words, the lowest period of anoestrus considered in this work was 14 days, corresponding to three consecutive samples under the threshold. In some ewes, more than one anoestrus period was observed (Fig. 1). TDA was defined for each ewe as the sum of days in anoestrus, considering all the anoestrus periods of that ewe. Four consecutive samples with progesterone levels higher or equal than the threshold was considered an occurrence compatible with normal cyclicity. Conversely, ewes with more than 4 consecutive samples higher or equal than the threshold were considered pathological (i.e., persistent corpus luteum) and were not considered in this study. Likewise, ewes not cycling in the preceeding breeding season, based on three samples one week apart taken in October and ewes with progesterone levels under the threshold in all samples taken in January, were not considered.

The second reproductive seasonality trait was the progesterone cycling months (P4CM), defined for each ewe as the rate of cycling months between January and August based on progesterone determinations. A ewe was considered cycling in a particular month when progesterone level was higher or equal than 0.5 ng/ml in at least one blood sample in that month. Cycling and non-cycling ewes in each month were coded as “1” and “0”, respectively. In this way, P4CM for each ewe could range from 0.125 (1/8) to 1 (8/8).

Finally, the third reproductive seasonality trait considered was the oestrus cycling months (OCM), defined for each ewe as the rate of months cycling between January and August based on oestrus records. Eight vasectomised rams fitted with harnesses and marking crayons were joined with the ewes and daily oestrus detection was performed [32]. Oestrus was recorded as a colour mark on the rump of the ewes, easy to identify visually. As ovulatory cycles occur approximately every 17 days, the colour of the marker crayon was changed every 2 weeks to avoid confusing marks between consecutive oestrus. A ewe was considered cycling in a particular month when at least one oestrus mark was detected in that month. Oestrus records in days with progestrone concentrations higher or equal than the threshold were not considered. Oestrus records followed by progesterone concentrations lower than the threshold in the next two sampling dates were not

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