INTRODUCTION

Small ruminants that live at temperate latitudes utilize the photoperiod as a temporal signal to initiate changes in their reproductive status (Bittman, Karsch, & Hopkins, 1983). Changes in day length are perceived and translated into a physiological signal by the pineal gland through the night-time secretion of melatonin (Reiter, 1980). In mammalian reproduction, melatonin has a particular effect on ovaries by stimulating receptor sites within the hypothalamus, pituitary and gonadal axis (Carla Cristina et al., 2013; Malpaux, Daveau, Maurice-Mandon, Duarte, & Chemineau, 1998). Melatonin action is mediated through specific receptors. In mammals, there are two

Polymorphisms of the melatonin receptor 1A gene that affects the reproductive seasonality and litter size in Small Tail Han sheep

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Abstract

Previous researches have shown that MTNR1A plays an essential role in sheep reproduction. However, most researches focused more on the reproductive seasonality of sheep, and few scientists had studied the association of polymorphisms of the MTNR1A gene with ovine litter size and reproductive seasonality. Therefore, we chose MTNR1A gene to detect its novel sequence polymorphisms and population genetics and analyse their association with seasonal reproduction and litter size in ewes. The mRNA expression level in hypothalamus, pituitary and ovary was also detected. In this study, five polymorphisms (g.15118664G > T, g.15118683C > T, g.15118756C > T, g.15118774C > T and g.15118951G > A) were identified in exon 2. Most importantly, the g.15118683C > T and g.15118951G > A were significant difference between year-round oestrous sheep and seasonal oestrous sheep (p < .01), and g.15118756C > T had a great effect on litter size of Small Tail Han sheep (p < .05). In addition, the mRNA expression level of MTNR1A in the hypothalamus of polytocous Small Tail Han sheep was significantly higher than that in monotocous Small Tail Han sheep (p < .01) and the expression of MTNR1A in the hypothalamus of year-round oestrous sheep was significantly higher than that in seasonal oestrous sheep (p < .01). Polymorphisms in exon 2 may regulate the reproductive seasonality and litter size of ewes by influencing gene expression to regulate the reproductive seasonality and litter size of ewes. Our studies provided useful guidance in marker-assisted selection of the litter size in Small Tail Han sheep.

KEYWORDS
litter size, reproductive seasonality, sheep, SNPs

1 | INTRODUCTION

Small ruminants that live at temperate latitudes utilize the photoperiod as a temporal signal to initiate changes in their reproductive status (Bittman, Karsch, & Hopkins, 1983). Changes in day length are perceived and translated into a physiological signal by the pineal gland through the night-time secretion of melatonin (Reiter, 1980). In mammalian reproduction, melatonin has a particular effect on ovaries by stimulating receptor sites within the hypothalamus, pituitary and gonadal axis (Carla Cristina et al., 2013; Malpaux, Daveau, Maurice-Mandon, Duarte, & Chemineau, 1998). Melatonin action is mediated through specific receptors. In mammals, there are two
high-affinity melatonin receptor subtypes, MTNR1A and MTNR1B, but only the MTNR1A seems to be involved in the regulation of reproductive activity (Carcangiu et al., 2011; Mura et al., 2014). With the deepening of researches, several studies have found the relationships between MTNR1A and reproduction activity in different animal species (Alsiddig et al., 2016; Carcangiu, Vaccà, et al., 2009; Zetouni et al., 2014). In chicken, melatonin receptor subtypes were identified in ovaries, suggesting that melatonin directly affects ovarian function through activation of multiple receptors (Sundaresan et al., 2009). In geese, the expression levels of MTNR1A initially increased and later decreased during the follicular development cycle, indicating that melatonin receptors participated in activating small white follicles and small yellow follicles to develop into subsequent greater hierarchical follicles (He et al., 2014). In sheep, most studies were centred on the positions of 606 and 612 in the MTNR1A gene exon 2, whose mutation could result in association with seasonal reproduction (Chu, Cheng, Liu, Fang, & Ye, 2006; Giantsis, Laliotis, Stoupà, & Avdi, 2016; Luridiana et al., 2015; Pelletier et al., 2000). Polymorphisms within the MTNR1A gene in the Sarda sheep breed, which exhibits an anoestrous period in late winter/spring, led to advances in reproductive resumption (Carcangiu, Vaccà, et al., 2009). In Dorset ewe lambs, the polymorphisms at this gene were shown to influence the beginning of puberty (Mateescu, Lunsford, & Thonney, 2009). In the Aragonesa breed, however, only the polymorphism in position of 612 was associated with a greater percentage of oestrous cyclic ewes between January and August (Martínez-Royo, Lahoz, Alabart, Folch, & Calvo, 2012). In the Ile de France ewes, the polymorphism in position of 606 was not associated with a difference in the onset, cessation or length of the breeding season among the animals of the two homozygous genotypes (Hernandez et al., 2005). Thus, numerous studies have investigated the relationship between MTNR1A and reproduction traits in different species and made it as a potential candidate gene for QTLs. However, these results in sheep also indicated that the relationship between the MTNR1A gene polymorphism and the reproduction can be varied with the breed.

As we all know, the prolificacy of sheep is an important economic trait. Generally, the litter size in the first parity is relatively lower, and the previous study indicated that the average litter size of four Swiss sheep breeds ranged from 1.36 to 1.57 in the first parity, from 1.52 to 1.75 in the second parity and from 1.56 to 1.86 in the third parity (Hagger, 2002). In the Hu sheep, the litter size in the first parity averages 1.72 and second parity litter size is 2.17 (Guan et al., 2011). Small Tail Han sheep, a famous Chinese indigenous breed for its prolificacy, average litter size is 2.67 (Guo, 2018). However, previous researches on the mechanism of melatonin regulation of animal reproduction have mainly focused on the reproductive seasonality in hypothalamus and pituitary (Gunwant et al., 2018), although there were some studies that have shown the expression profile of MTNR1A in the ovine ovaries in different sheep breeds (Jiang et al., 2017; Martine, Agnès, & Beno, 2005), a few studies have shown that the polymorphism of the MTNR1A gene is significantly related to the litter size of ewes (Chu, Cheng, Liu, Fang, & Ye, 2008; Wang, 2013).

Therefore, this experiment aimed to detect the SNPs in MTNR1A and, subsequently, analyse polymorphisms in 6 different Chinese native sheep breeds, and explored their association with ovine reproduction traits (litter size) in addition to the reproductive seasonality. The expression levels of MTNR1A gene in 3 major tissues controlling reproduction at different fecundity sheep were also conducted. Our study aimed to identify a genetic marker conceivably valuable for marker-assisted selection.

## 2 MATERIALS AND METHODS

### 2.1 Animals preparation, sample collection and DNA extraction

All the experimental procedures mentioned in the present study were approved by the Science Research Department (in charge of animal welfare issue) of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (IAS-CAAS) (Beijing, China). Ethical approval on animal survival was given by the animal ethics committee of IAS-CAAS (No. IASCAAS-AE-03, 12 December 2016).

As detailed in Table 1, 737 ewes from six sheep breeds were selected for genotyping. Jugular vein blood samples were collected for DNA extraction using the phenol–chloroform method and then dissolved in ddH2O.

### 2.2 Primer design and genotyping

The primers for genotyping were designed using MassARRAY Assay Design v3.1 from Beijing Compass Biotechnology Co., Ltd. According to

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**Table 1** Information of six sheep breeds selected for genotyping

| Breed                  | Number | Type                             | District                                      |
|------------------------|--------|----------------------------------|-----------------------------------------------|
| Small Tail Han sheep   | 380    | Polyembryony and year-round oestrus | Yuncheng, Shandong Province, China            |
| Hu sheep               | 101    | Polyembryony and year-round oestrus | Xuzhou, Jiangsu Province, China               |
| Cele black sheep       | 52     | Polyembryony and year-round oestrus | Cele, Hetian, Xinjiang Uygur Autonomous Region, China |
| Prairie Tibetan sheep  | 161    | Single birth and seasonal oestrus | Dangxiong, Tibet Autonomous Region, China     |
| Sunite sheep           | 21     | Single birth and seasonal oestrus | Wulate Zhongqi, Bayanmaoer, Inner Mongolia Autonomous Region, China |
| Tan sheep              | 22     | Single birth and seasonal oestrus | Yanchi, Ningxia Hui Autonomous Region, China |
the sheep MTNR1A sequence (GenBank accession no. NC_019483.2). Genotyping results were validated by PCR and sequencing. The qPCR primers were designed using primer 3 software (http://bioinfo.ut.ee/prime r3‐0.4.0/) and sheep MTNR1A mRNA (GenBank accession no. NM_001009725.1) and ACTB mRNA sequences (GenBank accession no. NM_001009784). All primers were synthesized by Beijing Tianyi Huiyuan Biotechnology Co. Ltd. Primers’ information is listed in Table 2. Five loci in the MTNR1A gene (g.15118664G > T, g.15118683C > T, g.15118756C > T, g.15118774C > T and g.15118951G > A) were selected for genotyping in 737 samples from Small Tail Han, Hu, Cele black, Prairie Tibetan, Sinite and Tan sheep. Genotyping was performed using a MassARRAY® SNP analysis (http://www.sequenom.com) (Johansen, Andersen, Børsting, & Morling, 2013). The polymerase chain reaction system and temperature were described in detail in a previous study (Zhou et al., 2018). Only those samples with a >95% success rate and only those SNPs with a genotype success rate of >95% were included in the analysis.

2.3 | RNA extraction, cDNA synthesis and qPCR

According to the litter size and the oestrous characters (Zhou et al., 2018), three ewes under each group were selected for expression study. RNA was extracted from three tissues (hypothalamus, pituitary and ovary) which are especially vital for mammal reproduction using the RNAprep Pure Tissue Kit (Tiangen). Quantity and quality of total RNA were determined using a NanoDrop 2000 and 1.2% agarose gel electrophoresis. The cDNAs were synthesized by PrimeScript™ RT Reagent Kit (TaKaRa) according to the kit instructions. The qPCR was performed on a Roche LightCycler® 480II (Roche) and carried out in 20 μl containing 10 μl SYBR Premix Ex Taq II (TaKaRa), 0.8 μl each primer, 2 μl cDNA and 6.4 μl ddH₂O. The qPCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C for 30 s. ACTB was used as a reference gene.

2.4 | Statistical analysis

Genotype and allele frequency, polymorphism information content (PIC), heterozygosity (HE) and effective number of alleles (NE) were calculated. Then, the distributions of genotypes for each SNP in the studied populations were tested for deviation from Hardy–Weinberg equilibrium by the Hardy–Weinberg law. Statistical analyses were performed using SAS (V. 9.2) (SAS Institute Inc.). Differences among three groups of samples were tested by the least significant difference test. p values <.05 were considered to be significant. The adjusted linear model was: $y_{ijn} = \mu + P_i + G_j + I_{PG} + e_{ijn}$, where $y_{ijn}$ is the phenotypic value of litter size; $\mu$ is the population mean; $P_i$ is the fixed effect of the $i$th parity ($i = 1, 2, 3$); $G_j$ is the fixed effect of the $j$th genotype ($j = 1, 2, 3$); $I_{PG}$ is the interaction effect of parity and genotype; and $e_{ijn}$ is the random residual. The gene expression data were normalized to the reference gene ACTB, and relative

| TABLE 2 | Primers information |
|---|---|---|
| Primer name | Sequences (5’−3’) | Product size | Usage |
| MTNR1A-1-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 99bp | PCR for g.15118664G > T |
| MTNR1A-1-R | ACGTTGAGTTAATCAGCCACAAACAGCC | 99bp | PCR for g.15118664G > T |
| MTNR1A-1-E | CATCCTGGGTCCATGCTG | Extension reaction |
| MTNR1A-2-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 116bp | PCR for g.15118683C > T |
| MTNR1A-2-R | ACGTTGAGTTAATCAGCCACAAACAGCC | 116bp | PCR for g.15118683C > T |
| MTNR1A-2-E | CTGACACTTATTGGTCTCT | Extension reaction |
| MTNR1A-3-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 99bp | PCR for g.15118756C > T |
| MTNR1A-3-R | ACGTTGAGTTAATCAGCCACAAACAGCC | 99bp | PCR for g.15118756C > T |
| MTNR1A-3-E | CCAACAAACATGGTACCAAATT | Extension reaction |
| MTNR1A-4-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 112bp | PCR for g.15118774C > T |
| MTNR1A-4-R | ACGTTGAGTTAATCAGCCACAAACAGCC | 112bp | PCR for g.15118774C > T |
| MTNR1A-4-E | TTGACACGGACAAAACACGGAAAC | Extension reaction |
| MTNR1A-5-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| MTNR1A-5-R | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| MTNR1A-5-E | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| MTNR1A-6-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| MTNR1A-6-E | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| MTNR1A-7-F | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| MTNR1A-7-R | ACGTTGAGTTAATCAGCCACAAACAGCC | 117bp | PCR for g.15118954G > A |
| ACTB-F | GCGTGATTCCCCTCCATCGT | qPCR |
| ACTB-R | GCGTGATTCCCCTCCATCGT | qPCR |

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expression level was calculated by the 2^ΔΔCt method (Schmittgen & Livak, 2008).

3 | RESULTS

3.1 | Polymorphisms of the coding region of the MTNR1A gene in Small Tail Han sheep

In this study, 5 SNPs in the exon 2 of the MTNR1A gene were identified by sequencing amplicon using primer 6 (MTNR1A-6-F and MTNR1A-6-R) in Table 2. Sequencing peak of different genotypes of the 5 SNPs (g.15118664G > T, g.15118683C > T, g.15118756C > T, g.15118774C > T and g.15118951G > A) is shown in Figure 1. The basic information and allele frequencies in Small Tail Han sheep are shown in Figure 2. Two SNPs (g.15118664G > T and g.15118683C > T) were identified as those involved in amino acid changes. The chi-square test demonstrated that all SNPs were under Hardy–Weinberg equilibrium (p > .05).

3.2 | Population genetic analysis of polymorphism in the MTNR1A gene

Besides Small Tail Han sheep (Figure 2), population genetic characteristics of 5 SNPs in the other 5 sheep breeds were also analysed, and the results are listed in Table 3. It revealed that g.15118664G > T and g.15118951G > A loci were moderately polymorphic (0.25 < PIC < .5), while others were at a low rate of polymorphisms in Small Tail Han sheep (Figure 2e). The g.15118756C > T and g.15118774C > T in Sunite sheep as well as g.15118951G > A locus in Hu sheep, Prairie Tibetan sheep, Cele black sheep and Tan sheep were moderately polymorphic (0.25 < PIC < .5). The chi-square test indicated that all SNPs were under Hardy–Weinberg equilibrium (p > .05) except g.15118683C > T in Tan sheep. In addition, we classified six breeds into two categories, year-round oestrous and seasonal oestrous, based on the oestrous characteristics, and the results of comparison of the population genetic analysis are shown in Table 4. The results indicated that the g.15118683C > T and g.15118951G > A were significantly different between year-round oestrous sheep and seasonal oestrous sheep (p < 0.01).

3.3 | Association of polymorphisms of 5 loci with litter size in Small Tail Han sheep

The results of association analysis between 5 loci with litter size in Small Tail Han sheep are shown in Table 5. At g.15118756C > T, sheep with TT genotype had a large litter size than those with CC and CT genotype (p < .05). At other loci, no significant differences in litter size between different genotypes were found. However, it can be seen that the mutant homozygotes were higher than the other two genotypes in g.15118664G > T, g.15118683C > T, g.15118774C > T and g.15118951G > A, although it does not reach a significant level.

3.4 | Expression of MTNR1A in sheep with different oestrous characters and fecundity

The expression of MTNR1A gene in hypotalamus, pituitary and ovary tissues in polytocous Small Tail Han sheep and monotocous Small Tail Han sheep was detected. The result is shown in Figure 3. MTNR1A gene was expressed in all three tissues with the highest level in the hypothalamus, followed by pituitary and ovary. The expression of MTNR1A in the hypothalamus of polytocous Small Tail Han sheep was significantly higher than that in monotocous Small Tail Han sheep (p < .01); it also reached a significant level in ovary (p < .05). However, there was no significant difference in pituitary (p > .05). The expression of MTNR1A gene in hypothalamus, pituitary and ovary tissues in seasonal oestrous Sunite sheep and year-round oestrous Small Tail Han sheep is also shown in Figure 3. MTNR1A gene was expressed in all three tissues with the highest level in the hypothalamus, followed by pituitary and ovary. The expression of MTNR1A in the hypothalamus of Small Tail Han sheep was significantly higher than that in Sunite sheep (p < .01); it also reached a significant level in the pituitary (p < .05). However, there was no significant difference in the ovary in two breeds (p > .05).

4 | DISCUSSION

4.1 | Polymorphism of MTNR1A gene

Polymorphisms of MTNR1A gene have a great influence on the reproduction of goat (Carcangiu, Vacca, et al., 2009; Chu et al., 2007), cattle (Elraey et al., 2011), pig (Ramírez et al., 2009), goose (Alsiddig et al., 2016), buffalo (Gunwant et al., 2018; Jiang et al., 2017), chicken (Sundaresan et al., 2009) and sheep (Pelletier et al., 2000, Chu, Ji, & Chen, 2003, Chu et al., 2006, Chu et al., 2008, Mateescu et al., 2009, Wang, 2013, Lei, Di, Liu, & Chu, 2015, Giantsis et al., 2016, Calvo et al., 2018). The earliest researchers found two polymorphic loci in the 824bp of exon 2 of the MTNR1A gene in many sheep breeds using restriction endonucleases MnlI and RsaI (Messer et al., 1997; Notter, Cockett, & Hadfield, 2003). In our previous studies, these two restriction enzymes were used to digest 824bp of exon 2 in year-round oestrous and seasonal oestrous sheep, and the two sites were all found (Chu et al., 2006, 2003; Ji, Chu, Chen, & Zhu, 2003). Subsequently, A. Martínez-Royo found 11 SNPs in the coding region by sequencing, in which 9 SNPs (g.15119131G > A, g.15118951G > A, g.15118882G > T, g.15118851C > T, g.15118774C > T, g.15118666G > A, g.15118664G > T, g.15118464G > A and g.15118428T > C) were detected in Hu sheep:
interestingly, these sites are all on exon 2 (Martínez-Royo et al., 2012; Wang, Deng, et al., 2015). In the present study, we firstly carried out polymorphism detection in exon 2, the most widely studied exon of MTNR1A, and found five SNPs in sheep, each locus contained three genotypes (Figure 1), and both g.15118664G > T and g.15118683C > T loci were involved in amino acid changes (Figure 2b). Interestingly, three of the five loci had been detected in Altay sheep and Hu sheep (Wang, Shi, et al., 2015), and the other two loci were also found in the Rasa Aragonesa sheep (Calvo et al., 2018).

However, the researchers did not mention whether these loci play biological functions in ovine reproduction. In order to better understand the functions of the loci, we conducted population genetic analysis of five loci in six sheep breeds according to the typing results. Except for g.15118664G > T and g.15118951G > A loci, all others were at a low rate of polymorphisms in Small Tail Han sheep. In other five breeds, only the g.15118756C > T and g.15118774C > T in Sunite sheep as well as g.15118951G > A locus in Hu sheep, Prairie Tibetan sheep, Cele black sheep and Tan sheep were moderately polymorphic (.25 < PIC < .5). These results indicated that different genotypes of five loci were widely found in various sheep breeds, which provides valuable information to further study their functions.

![FIGURE 2](image_url)

**FIGURE 2** Polymorphisms of the coding region (exon 2) of the MTNR1A gene in Small Tail Han sheep. (a) The detected SNPs and specific primers. A total of 5 SNPs are found in the exon 2 of MTNR1A gene; (b) the change of amino acids; (c–d) the genotype and allele frequencies of the MTNR1A gene; (e) the test of polymorphism information content (PIC), heterozygosity (HE) and Hardy–Weinberg equilibrium (HWE)
Reproductive seasonality and MTNR1A gene

MTNR1A, a receptor of melatonin, plays important roles in regulating the physiological rhythm and reproductive seasonality of mammals (Lei et al., 2015). Many researches indicated that polymorphisms of MTNR1A gene had effects on the reproductive seasonality in sheep (Calvo et al., 2018; Carcangiu, Mura, et al., 2009; Chu et al., 2006; Giantsis et al., 2016; Mateescu et al., 2009; Pelletier et al., 2000), goat (Carcangiu, Vacca, et al., 2009; Chu et al., 2007) and buffalo (Gunwant et al., 2018; Luridiana et al., 2012). MTNR1A is one potential candidate gene involved in the control of ovine reproductive seasonality. The previous studies found homozygous genotype for the polymorphic MnlI site at position 605 of exon 2 was associated with year-round oestrus in ewes (Pelletier et al., 2000); if not, this site was associated with seasonal anovulatory activity in ewes (Chu et al., 2006; Notter et al., 2003). Besides, another position of 612 (exon 2) showed an increase in the percentage of oestrous cyclic ewes (Martínez-Royo et al., 2012) and influenced spring reproductive resumption in the Sarda sheep breed (Mura et al., 2014). In our study, we divided the six sheep breeds into two groups (year-round oestrous sheep and seasonal oestrous sheep) according to the oestrous characteristics and found that genotype frequency and allele frequency were significantly different between year-round oestrous sheep and seasonal oestrous sheep (p < .01) in the g.15118683C > T and g.15118951G > A loci for the first time. The results preliminarily showed that they may be related to the oestrus or reproductive seasonality and play important roles in year-round oestrous sheep. However, the similar

### TABLE 3 Population genetic analysis of 5 loci of MTNR1A gene in five sheep breeds

| Locus         | Breed              | Genotype frequency | Allele frequency | PIC  | HE   | NE   | Chi-square test (p-value) |
|---------------|--------------------|--------------------|------------------|------|------|------|--------------------------|
| g.15118664G > T | Hu sheep           | GG                 | 0.81             | 0.90 | 0.10 | 0.16 | 0.18 1.22 .99            |
|               | Prairie Tibetan sheep | GT                 | 0.18             | 0.01 | 0.10 | 0.16 | 0.18 1.22 .99            |
|               | Cele black sheep   | TT                 | 0.01             | 0.10 | 0.16 | 0.16 | 0.18 1.22 .99            |
|               | Sinite sheep       | G                  | 0.90             | 0.10 | 0.16 | 0.16 | 0.18 1.22 .99            |
|               | Tan sheep          | T                  | 0.16             | 0.18 | 0.10 | 0.16 | 0.18 1.22 .99            |
| g.15118683C > T | Hu sheep           | CC                 | 0.85             | 0.93 | 0.07 | 0.13 | 0.14 1.16 .42            |
|               | Prairie Tibetan sheep | CT                 | 0.15             | 0.03 | 0.07 | 0.13 | 0.14 1.16 .42            |
|               | Cele black sheep   | TT                 | 0.00             | 0.96 | 0.04 | 0.06 | 0.07 1.08 .77            |
|               | Sinite sheep       | C                  | 0.96             | 0.04 | 0.06 | 0.06 | 0.07 1.08 .77            |
|               | Tan sheep          | T                  | 0.06             | 0.94 | 0.02 | 0.05 | 0.06 1.05 .91            |
| g.15118756C > T | Hu sheep           | CC                 | 0.80             | 0.90 | 0.10 | 0.17 | 0.19 1.23 .92            |
|               | Prairie Tibetan sheep | CT                 | 0.19             | 0.09 | 0.10 | 0.17 | 0.19 1.23 .92            |
|               | Cele black sheep   | TT                 | 0.01             | 0.92 | 0.08 | 0.06 | 0.07 1.08 .77            |
|               | Sinite sheep       | C                  | 0.92             | 0.08 | 0.06 | 0.06 | 0.07 1.08 .77            |
|               | Tan sheep          | T                  | 0.06             | 0.95 | 0.02 | 0.05 | 0.06 1.05 .91            |
| g.15118774C > T | Hu sheep           | CC                 | 0.80             | 0.90 | 0.10 | 0.17 | 0.19 1.23 .92            |
|               | Prairie Tibetan sheep | CT                 | 0.19             | 0.09 | 0.10 | 0.17 | 0.19 1.23 .92            |
|               | Cele black sheep   | TT                 | 0.01             | 0.92 | 0.08 | 0.06 | 0.07 1.08 .77            |
|               | Sinite sheep       | C                  | 0.92             | 0.08 | 0.06 | 0.06 | 0.07 1.08 .77            |
|               | Tan sheep          | T                  | 0.06             | 0.95 | 0.02 | 0.05 | 0.06 1.05 .91            |
| g.15118951G > A | Hu sheep           | GG                 | 0.44             | 0.63 | 0.37 | 0.36 | 0.47 1.88 .08            |
|               | Prairie Tibetan sheep | GA                 | 0.38             | 0.63 | 0.37 | 0.36 | 0.47 1.88 .08            |
|               | Cele black sheep   | AA                 | 0.18             | 0.63 | 0.37 | 0.36 | 0.47 1.88 .08            |
|               | Sinite sheep       | G                  | 0.30             | 0.57 | 0.43 | 0.37 | 0.47 1.88 .08            |
|               | Tan sheep          | A                  | 0.16             | 0.57 | 0.43 | 0.37 | 0.47 1.88 .08            |

Note: PIC, HE and NE represent polymorphism information content, heterozygosity and effective number of alleles, respectively; p > .05 indicates the locus was under Hardy–Weinberg equilibrium.

4.2 Reproductive seasonality and MTNR1A gene

MTNR1A, a receptor of melatonin, plays important roles in regulating the physiological rhythm and reproductive seasonality of mammals (Lei et al., 2015). Many researches indicated that polymorphisms of MTNR1A gene had effects on the reproductive seasonality in sheep (Calvo et al., 2018; Carcangiu, Mura, et al., 2009; Chu et al., 2006; Giantsis et al., 2016; Mateescu et al., 2009; Pelletier et al., 2000), goat (Carcangiu, Vacca, et al., 2009; Chu et al., 2007) and buffalo (Gunwant et al., 2018; Luridiana et al., 2012). MTNR1A is one potential candidate gene involved in the control of ovine reproductive seasonality. The previous studies found homozygous genotype for the polymorphic MnlI site at position 605 of exon 2 was associated with year-round oestrus in ewes (Pelletier et al., 2000); if not, this site was associated with seasonal anovulatory activity in ewes (Chu et al., 2006; Notter et al., 2003). Besides, another position of 612 (exon 2) showed an increase in the percentage of oestrous cyclic ewes (Martínez-Royo et al., 2012) and influenced spring reproductive resumption in the Sarda sheep breed (Mura et al., 2014). In our study, we divided the six sheep breeds into two groups (year-round oestrous sheep and seasonal oestrous sheep) according to the oestrous characteristics and found that genotype frequency and allele frequency were significantly different between year-round oestrous sheep and seasonal oestrous sheep (p < .01) in the g.15118683C > T and g.15118951G > A loci for the first time. The results preliminarily showed that they may be related to the oestrus or reproductive seasonality and play important roles in year-round oestrous sheep. However, the similar
effects in other 3 loci were not observed, which was completely consistent with previous studies, possibly because these mutations were not in the transmembrane region (Martínez-Royo et al., 2012; Wang, Deng, et al., 2015). Whether those 3 mutations affect ovine seasonal oestrus and other reproductive traits by altering the structure of MTNR1A requires further researches.

### 4.3 Litter size and polymorphism of MTNR1A gene

The previous studies also found that the polymorphisms of MTNR1A gene may affect the litter size in Small Tail Han sheep (Chu et al., 2008) and pig (Wang et al., 2006). Although these studies were not very clear, it also provides a new research direction. In the recent years, studies have found that MTNR1A gene could alter the number of lambing in ewes indirectly through influencing pregnancy (Luridiana et al., 2014), or involved in the production of eggs in birds (Alsiddig et al., 2016; Feng et al., 2018). Present association analysis revealed that mutation at g.15118756C > T had a great effect on litter size (the first, second and third parity) in Small Tail Han sheep ($p < .05$) (Table 5), which indicated that polymorphism of ovine MTNR1A gene may play a fundamental role in litter size of sheep. More interestingly, it could be seen the mutant homozygotes

### TABLE 4 Frequencies of 5 loci in MTNR1A gene in sheep with different oestrous characters

| Locus          | Characteristics of oestrus | Genotype frequency | Allele frequency | Chi-square test (P-value) |
|----------------|----------------------------|--------------------|------------------|---------------------------|
| g.15118664G > T | Year-round oestrous sheep  | GG 0.83            | G 0.88           | 0.71                      |
|                | Seasonal oestrus           | GT 0.09            | T 0.12           |                           |
|                |                            | TT 0.08            |                 |                           |
| g.15118683C > T| Year-round oestrous sheep  | CC 0.85            | C 0.93           | 7.22E–07                  |
|                | Seasonal oestrus           | CT 0.15            | T 0.07           |                           |
|                |                            | TT 0.00            |                 |                           |
| g.15118756C > T| Year-round oestrous sheep  | CC 0.82            | C 0.90           | 0.93                      |
|                | Seasonal oestrus           | CT 0.17            | T 0.10           |                           |
|                |                            | TT 0.01            |                 |                           |
| g.15118774C > T| Year-round oestrous sheep  | CC 0.82            | C 0.90           | 0.97                      |
|                | Seasonal oestrus           | CT 0.17            | T 0.10           |                           |
|                |                            | TT 0.02            |                 |                           |
| g.15118951G > A| Year-round oestrous sheep  | GG 0.73            | G 0.86           | 0.60E–03                  |
|                | Seasonal oestrus           | GA 0.25            | T 0.14           |                           |
|                |                            | AA 0.11            |                 |                           |

### TABLE 5 LSM ± SE of litter size in Small Tail Han sheep with different genotypes

| Locus          | Genotype | Litter size of the first parity | Litter size of the second parity | Litter size of the third parity |
|----------------|----------|---------------------------------|----------------------------------|--------------------------------|
| g.15118664G > T| GG       | 2.17 ± 0.05 (267)               | 2.34 ± 0.06 (255)                | 2.83 ± 0.09 (99)                |
|                | GT       | 2.04 ± 0.10 (77)                | 2.28 ± 0.11 (72)                 | 2.80 ± 0.17 (30)                |
|                | TT       | 2.67 ± 0.37 (6)                 | 2.95 ± 0.37 (6)                  | 3.00 ± 0.52 (3)                 |
| g.15118683C > T| CC       | 2.15 ± 0.06 (272)               | 2.31 ± 0.06 (260)                | 2.76 ± 0.09 (97)                |
|                | CT       | 2.10 ± 0.10 (78)                | 2.33 ± 0.11 (72)                 | 2.97 ± 0.16 (34)                |
|                | TT       | 2.67 ± 0.52 (13)                | 2.77 ± 0.53 (6)                  | 3.00 ± 0.64 (3)                 |
| g.15118756C > T| CC       | 2.18 ± 0.06b (253)              | 2.26 ± 0.06b (244)               | 2.67 ± 0.11b (96)               |
|                | CT       | 1.99 ± 0.09b (82)               | 2.23 ± 0.11b (75)                | 2.64 ± 0.19b (31)               |
|                | TT       | 2.86 ± 0.32a (7)                | 3.08 ± 0.34a (5)                 | 3.30 ± 0.52a (3)                |
| g.15118774C > T| CC       | 2.19 ± 0.06 (260)               | 2.36 ± 0.06 (249)                | 2.84 ± 0.09 (99)                |
|                | CT       | 2.02 ± 0.10 (84)                | 2.22 ± 0.10 (78)                 | 2.74 ± 0.16 (31)                |
|                | TT       | 2.67 ± 0.37 (6)                 | 2.69 ± 0.37 (5)                  | 3.00 ± 0.52 (3)                 |
| g.15118951G > A| GG       | 2.14 ± 0.07 (152)               | 2.36 ± 0.08 (145)                | 2.72 ± 0.12 (54)                |
|                | GA       | 2.18 ± 0.08 (141)               | 2.28 ± 0.08 (133)                | 2.95 ± 0.13 (57)                |
|                | AA       | 2.05 ± 0.14 (41)                | 2.28 ± 0.15 (39)                 | 2.69 ± 0.23 (16)                |

Note: LSM, least squares mean; SE, standard error; numbers in the parentheses next to litter size represent the amount of sheep of each genotype; different small letters in the same group mean significant difference ($p < .05$).
were higher than the other two genotypes in g.15118664G > T, g.15118683C > T and g.15118774C > T in the first parity, although it does not reach a significant level, as well as in the second or third parity in above 3 loci (Table 5). The reason for this may be the limited sample size, and we will use larger samples to further verify the functions of those loci. Collectively, the results of the current study suggested that the polymorphisms of ovine MTNR1A gene play an important role in litter size of ewes; SNPs in this gene could be used as markers in marker-assisted selection for ovine reproductive traits.

4.4 | Expression of MTNR1A in sheep with different fecundity and different oestrous characters

Melatonin exerts its role as the regulator of reproductive activity by virtue of variation in its nocturnal secretion from the pineal gland. The reception of these signals by neuroendocrine cells is mediated through melatonin receptors of MTNR1A and MTNR1B, but it is MTNR1A which is chiefly associated with regulation of ovine seasonal reproductive activity (Saxena, Jha, Meena, & Naqvi, 2014), and most studies found MTNR1A plays a vital role in hypothalamus, pituitary and ovary tissues (Carla Cristina et al., 2013; Lei et al., 2015) and MTNR1A was expressed in hypothalamic–pituitary–ovarian axis tissues with entire oestrous cycles in Ganjia Tibetan sheep (Yang et al., 2019). Based on the results of the association analysis of oestrus and litter size, we selected two comparison groups to analyse the expression of MTNR1A in hypothalamus, pituitary and ovary tissues; the expression tendency among the three tissues was completely consistent with previous studies (Guo, Liu, Zhang, & Wang, 2010; Jiang et al., 2017; Yang et al., 2019). Interestingly, the expression of MTNR1A in hypothalamus of polytocous Small Tail Han sheep was significantly higher than that in monotonous Small Tail Han sheep (p < .01), and this significant level was also achieved in the year-round oestrous Small Tail Han sheep and seasonal oestrous Sunite sheep (p < .01) (Figure 3). These results indicated a higher expression of MTNR1A in the hypothalamus, and MTNR1A may have a positive effect on litter size and year-round oestrus. Therefore, polymorphisms of ovine MTNR1A gene exon 2 may have effects on its expression in hypothalamus, which may further influence the reproductive seasonality and litter size of ewes.

5 | CONCLUSIONS

Both g.15118683C > T and g.15118951G > A of MTNR1A may affect the oestrus or reproductive seasonality in sheep breeds. The homozygous mutation (TT) in g.15118756C > T locus can significantly increase litter size in Small Tail Han sheep. The differential expression of MTNR1A in ovine hypothalamus with different fecundity or oestrous characters suggested that polymorphisms in exon 2 may influence gene expression to regulate the reproductive seasonality and litter size of ewes. Therefore, our studies could be useful in marker-assisted selection of the litter size in Small Tail Han sheep.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.
AUTHOR CONTRIBUTIONS

Mingxing Chu and Qiuyue Liu designed the research; Xiaoyun He and Zhuangbiao Zhang analysed or interpreted the data; Xiaoyun He drafted the paper.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

Alsididdig, M. A., Yu, S. G., Pan, Z. X., Widaa, H., Badri, T. M., Chen, J., & Liu, H. L. (2016). Association of single nucleotide polymorphism in melatonin receptor 1A gene with egg production traits in Yangzhou geese. Animal Genetics, 48, 245–249.

Bittman, E. L., Karsch, F. J., & Hopkins, J. W. (1983). Role of the pineal gland in ovine photoperiodism: Regulation of seasonal breeding and negative feedback effects of estradiol upon luteinizing hormone secretion. Endocrinology, 113, 329–336.

Calvo, J. H., Serrano, M., Martinez-Royo, A., Lahoz, B., Sarto, P., Ibañez-Deler, A., … Alabart, J. L. (2018). SNP rs403212791 in exon 2 of the MTNR1A gene is associated with reproductive seasonality in the Rasa Aragonesa sheep breed. Theriogenology, 113, 63–72. https://doi.org/10.1016/j.theriogenology.2018.02.013

Carcangiu, V., Mura, M. C., Pazzola, M., Vacca, G. M., Paludo, M., Marchi, B., … Luridiana, S. (2011). Characterization of the Mediterranean Italian buffaloes melatonin receptor 1A (MTNR1A) gene and its association with reproductive seasonality. Theriogenology, 76, 419–426. https://doi.org/10.1016/j.theriogenology.2011.02.018

Carcangiu, V., Mura, M. C., Vacca, G. M., Pazzola, M., Dettori, M. L., Luridiana, S., & Bini, P. P. (2009). Polymorphism of the melatonin receptor MT1 gene and its relationship with seasonal reproductive activity in the Sarda sheep breed. Animal Reproduction Science, 116, 65–72. https://doi.org/10.1016/j.anireprosci.2009.01.005

Carcangiu, V., Vacca, G. M., Mura, M. C., Dettori, M. L., Pazzola, M., Luridiana, S., & Bini, P. P. (2009). Relationship between MTNR1A melatonin receptor gene polymorphism and seasonal reproduction in different goat breeds. Animal Reproduction Science, 110, 71–78. https://doi.org/10.1016/j.anireprosci.2007.12.014

Carla Cristina, M., Fuchs, L. F. P., Ricardo Santos, S. E., Ricardo Martins, O. F., Manuel, D. J. S. E., Edmund Chada, B., & José Maria, S. (2013). Effects of melatonin on ovarian follicles. European Journal of Obstetrics & Gynecology and Reproductive Biology, 166, 178–184. https://doi.org/10.1016/j.ejogrb.2012.10.006

Chu, M. X., Cheng, D. X., Liu, W. Z., Fang, L., & Ye, S. C. (2006). Association between melatonin receptor 1A gene and expression of reproductive seasonality in sheep. Asian-Australasian Journal of Animal Sciences, 19, 1079–1084. https://doi.org/10.5713/ajas.2006.1079

Chu, M. X., Cheng, D. X., Liu, W. Z., Fang, L., & Ye, S. C. (2008). Association between exon 2 of melatonin receptor 1A gene and prolificacy in Small Tail Han sheep. Journal of Anhui Agricultural University, 35, 1–4.

Chu, M. X., He, Y. Q., Cheng, D. X., Ye, S. C., Fang, L., & Wang, J. Y. (2007). Association between expression of reproductive seasonality and alleles of melatonin receptor 1A in goats. Animal Reproduction Science, 101, 276–284. https://doi.org/10.1016/j.anireprosci.2006.09.012

Chu, M. X., Ji, C. L., & Chen, G. H. (2003). Association between PCR-RFLP of melatonin receptor 1a gene and high prolificacy in Small Tail Han sheep. Asian-Australasian Journal of Animal Sciences, 16, 1701–1704. https://doi.org/10.5713/ajas.2003.1701

Elrayy, M., Geshi, M., Somfai, T., Kaneda, M., Hirako, M., Abdelghaffar, A. E., … Nagai, T. (2011). Evidence of melatonin synthesis in the cumulus oocyte complexes and its role in enhancing oocyte maturation in vitro in cattle. Molecular Reproduction and Development, 78, 250–262. https://doi.org/10.1002/mrd.21295

Feng, P. S., Zhao, W. Q., Xie, Q., Zeng, T., Lu, I. Z., & Yang, L. (2018). Polymorphisms of melatonin receptor genes and their associations with egg production traits in Shaoxing duck. Asian-Australasian Journal of Animal Sciences, 31, 1535–1541. https://doi.org/10.5713/ajas.17.0828

Giantsis, I. A., Laliotis, G. P., Stoupas, O., & Avdi, M. (2016). Polymorphism of the melatonin receptor 1A (MNTR1A) gene and association with seasonality of reproductive activity in a local Greek sheep breed. Journal of Biological Research-Thessaloniki, 23, 9. https://doi.org/10.1186/s40709-016-0050-y

Guan, F., Pan, L., Li, J., Tang, H., Zhu, C., & Shi, G. Q. (2011). Polymorphisms of the prion protein gene and their effects on litter size and risk evaluation for scrapie in Chinese Hu sheep. Virus Genes, 43, 147–152. https://doi.org/10.1007/s11262-011-0609-5

Gunwant, P., Pandey, A. K., Kumar, A., Singh, L., Kumar, S., Phogat, J. B., … Magotra, A. (2018). Polymorphism of melatonin receptor (MTNR1A) gene and its association with seasonal reproduction in water buffalo (Bubalus bubalis). Animal Reproduction Science, 199, 51–59. https://doi.org/10.1016/j.anireprosci.2018.10.006

Guo, T. T., Liu, Y. Q., Zhang, Y. J., & Wang, X. (2010). The seasonal difference of MTNR1a gene mRNA expression in sheep. Journal of Gansu Agricultural University, 45, 14–15.

Guo, X. F. (2018). Study on molecular mechanism of FecB gene for fecundity in Small Tail Han Sheep. Doctoral Thesis. China Agricultural University, Beijing.

Hagger, C. (2002). Multitrait and repeatability estimates of random effects on litter size in sheep. Animal Science, 74, 209–216. https://doi.org/10.1017/S1357729800052371

He, H., Jiang, D. M., Kang, B., Ma, R., Bai, L., Wang, X., & Zhao, L. (2014). Gene expression profiling of melatonin receptor subtypes in the ovarian hierarchical follicles of the Sichuan white goose. Animal Reproduction Science, 145, 62–68. https://doi.org/10.1016/j.anireprosci.2013.12.012

Hernandez, X., Chesneau, B. D., Guillaume, D., Chemineau, P., Malpaux, B., & Migaud, M. (2005). Relationship between MT1 melatonin receptor gene polymorphism and seasonal physiological responses in Ile-de-France ewes. Reproduction, Nutrition, Development, 45, 151–162.

Ji, C. L., Chu, M. X., Chen, G. H., Zhou, G. L., & Zhu, Y. (2003). Polymorphism analysis for exon 2 of melatonin receptor 1a gene in four sheep breeds by PCR-RFLP. Journal of Huazhong Agricultural University, 22, 105–109.

Jiang, W. D., Mulatio, A., Zhang, J., Song, X. M., Li, L. R., & Shi, C. Q. (2017). Expression and sequence analysis of MTNR1a gene in Hetian sheep ovarian. Heilongjiang Animal Science and Veterinary Medicine, 12, 106–109.

Johansen, P., Andersen, J. D., Barsting, C., & Morling, N. (2013). Evaluation of the iPLEX® Sample ID Plus Panel designed for the Sequenom MassARRAY® system. A SNP typing assay developed for human identification and sample tracking based on the SNP for ID panel. Forensic Science International: Genetics, 7, 482–487. https://doi.org/10.1016/j.fsigen.2013.04.009

Lei, M. Y., Di, R., Liu, Q. Y., & Chu, M. X. (2015). Advance about molecular genetics of melatonin receptor 1A gene (MTNR1A). Journal of Agricultural Biotechnology, 23, 388–396.

Luridiana, S., Mura, M. C., Daga, C., Cosso, G., Bodano, S., Farci, F., … Carcangiu, V. (2014). Influences of melatonin treatment, melatonin...
receptor 1A (MTNR1A) and kisspeptin (KiSS-1) gene polymorphisms on first conception in Sarda ewe lambs. Reproduction, Fertility, and Development, 28, 750–756. https://doi.org/10.1071/RD14120
Luridiana, S., Mura, M. C., Daga, C., Diaz, M. L., Bini, P. P., Cosso, G., & Carcangi, V. (2015). The relationship between melatonin receptor 1A gene (MTNR1A) polymorphism and reproductive performance in Sarda breed sheep. Livestock Science, 171, 78–83. https://doi.org/10.1016/j.livsci.2014.11.004
Luridiana, S., Mura, M. C., Pazzola, M., Paludo, M., Cosso, G., Dettori, M. L., ... Carcangi, V. (2012). Association between melatonin receptor 1A (MTNR1A) gene polymorphism and the reproductive performance of Mediterranean Italian buffaloes. Reproduction, Fertility, and Development, 24, 983–987. https://doi.org/10.1071/RD11297
Malpau, B., Daveau, A., Maurice-Mandon, F., Durante, G., & Chemineau, P. (1998). Evidence that melatonin acts in the pre-mammillary hypothalamic area to control reproduction in the ewe: Presence of binding sites and stimulation of luteinizing hormone secretion by in situ microimplant delivery. Endocrinology, 139, 1508–1516.
Martine, M., Agnès, D., & Beno, T. M. (2005). MTNR1A melatonin receptors in the ovine pre-mammillary hypothalamus: Day-night variation in the expression of the transcripts. Biology of Reproduction, 72, 393–398.
Martínez-Royo, A., Lahoz, B., Alabart, J. L., Folch, J., & Calvo, J. H. (2012). Characterisation of the Melatonin Receptor 1A (MTNR1A) gene in the Rasa Aragonesa sheep breed: Association with reproductive seasonality. Animal Reproduction Science, 133, 169–175. https://doi.org/10.1016/j.anireprosci.2012.06.018
Mateescu, R. G., Lunsford, A. K., & Thonney, M. L. (2009). Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes. Journal of Animal Science, 87, 2485–2488.
Messer, L. A., Wang, L., Tuggle, C. K., Yerle, M., Chardon, P., Pomp, D., ... Notter, D. R. (1997). Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep, and cattle. Mammalian Genome, 8, 368–370.
Mura, M. C., Luridiana, S., Daga, C., Carcangi, V., Bodano, S., Cosso, G., ... Bini, P. P. (2014). Influence of melatonin receptor 1A gene polymorphisms on seasonal reproduction in Sarda ewes with different body condition scores and ages. Animal Reproduction Science, 149, 173–177. https://doi.org/10.1016/j.anireprosci.2014.07.022
Notter, D. R., Cockett, N. E., & Hadfield, T. S. (2003). Evaluation of melatonin receptor 1a as a candidate gene influencing reproduction in an autumn-lambing sheep flock. Journal of Animal Science, 81, 912–917. https://doi.org/10.2527/2003.814912x
Pelletier, J., Bodin, L., Hanoq, E., Malpau, B., Teysssier, J., Thimonier, J., & Chemineau, P. (2000). Association between expression of reproductive seasonality and alleles of the gene for Mel1a receptor in the ewe. Biology of Reproduction, 62, 1096–1101.
Ramírez, O., Tomás, A., Barragan, C., Nogueru, J. L., Amills, M., & Varona, L. (2009). Pig melatonin receptor 1a (MTNR1A) genotype is associated with seasonal variation of sow litter size. Animal Reproduction Science, 115, 317–322. https://doi.org/10.1016/j.anireprosci.2008.12.013
Reiter, R. J. (1980). The pineal and its hormones in the control of reproduction in mammals. Endocrine Reviews, 1, 109–131. https://doi.org/10.1210/edrv-1-2-109
Saxena, V. K., Jha, B. K., Meena, A. S., & Naqvi, S. M. K. (2014). Sequence analysis and identification of new variations in the coding sequence of melatonin receptor gene (MTNR1A) of Indian Chokla sheep breed. Meta Gene, 2, 450–458. https://doi.org/10.1016/j.mgene.2014.05.005
Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. Nature Protocols, 3, 1001–1008. https://doi.org/10.1038/nprot.2008.73
Sundaresan, N. R., Leo, M. D. M., Subramani, J., Anish, D., Sudhagar, M., Ahmed, K. A., ... Saxena, V. K. (2009). Expression analysis of melatonin receptor subtypes in the ovary of domestic chicken. Veterinary Research Communications, 33, 49–56. https://doi.org/10.1007/s11525-008-9071-9
Wang, Q. (2013). The gene polymorphism and expression research on candidate genes about high fecundity in three Xinjiang sheep breeds. Master Thesis, Xinjiang Agricultural University.
Wang, S. Y., Deng, S. Y., Yang, L. W., Gan, S. Q., Song, G. C., Shi, G. Q., & Zhang, W. (2015). Polymorphism of melatonin receptor 1A (MTNR1A) and the relationship with seasonal reproduction of sheep (Ovis aries). Acta Agriculturae Boreali-occidentalis Sinica, 24, 23–30.
Wang, S. Y., Shi, G. Q., Gan, S. Q., Song, G. C., Zhang, W., & Deng, S. Y. (2015). Expression change of MTNR1A gene in hypothalamus of Altay sheep (Ovis aries) at different reproductive stages and its relationship with seasonal reproduction. Journal of Southern Agriculture, 46, 1887–1892.
Wang, X. F., Wang, A. G., Fu, J. L., Li, J. Z., Han, L. P., & Yang, S. M. (2006). Effects of MTNR1A gene on litter size in a large white and a landrace herd. Hereditas (Beijing), 28, 805–809.
Yang, D. P., He, Y. Q., Ge, W. B., Zhang, Q. W., Cairang, H. D., Wang, X., ... Chen, W. G. (2019). Expression analysis of MTR1A gene in hypothalamic-pituitary-ovarian-axis during estrus cycle in Ganjia Tibetan sheep (Ovis aries). Journal of Agriculture Biotechnology, 27, 457–463.
Zetouni, L., Fonseca, P. D. D. S., Cardoso, D. F., Gil, F. M. M., Hurtado-Lugo, N. A., Aspilcueta-Borquis, R. R., ... Tonhati, H. (2014). Polymorphisms in the MTNR1A gene and their effects on the productive and reproductive traits in buffaloes. Tropical Animal Health and Production, 46, 337–340.
Zhou, M., Pan, Z. Y., Cao, X. H., Guo, X. F., He, X. Y., Sun, Q., ... Chu, M. X. (2018). Single nucleotide polymorphisms in the HIRA gene affect litter size in Small Tail Han Sheep. Animals, 8, 71. https://doi.org/10.3390/ani8050071

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