Genome-Wide Association Analyses Highlight the Potential for Different Genetic Mechanisms for Litter Size Among Sheep Breeds

Song-Song Xu1,2†, Lei Gao3,4†, Xing-Long Xie1,2, Yan-Ling Ren5, Zhi-Qiang Shen5, Feng Wang6, Min Shen3,4, Emma Eyþórsdóttir7, Jón H. Hallsson7, Tatyana Kiseleva8, Juha Kantanen9 and Meng-Hua Li1,2✉

Reproduction is an important trait in sheep breeding as well as in other livestock. However, despite its importance the genetic mechanisms of litter size in domestic sheep (Ovis aries) are still poorly understood. To explore genetic mechanisms underlying the variation in litter size, we conducted multiple independent genome-wide association studies in five sheep breeds of high prolificacy (Wadi, Hu, Icelandic, Finnsheep, and Romanov) and one low prolificacy (Texel) using the Ovine Infinium HD BeadChip, respectively. We identified different sets of candidate genes associated with litter size in different breeds: BMPR1B, FBN1, and MMP2 in Wadi; GRIA2, SMAD1, and CTNNB1 in Hu; NCOA1 in Icelandic; INHBB, NF1, FLT1, PTGS2, and PLCB3 in Finnsheep; ESR2 in Romanov and ESR1, GHR, ETS1, MMP15, FLI1, and SPP1 in Texel. Further annotation of genes and bioinformatics analyses revealed that different biological pathways could be involved in the variation in litter size of females: hormone secretion (FSH and LH) in Wadi and Hu, placenta and embryonic lethality in Icelandic, folliculogenesis and LH signaling in Finnsheep, ovulation and preovulatory follicle maturation in Romanov, and estrogen and follicular growth in Texel. Taken together, our results provide new insights into the genetic mechanisms underlying the prolificacy trait in sheep and other mammals, suggesting targets for selection where the aim is to increase prolificacy in breeding projects.

Keywords: sheep, prolificacy, genome-wide association study, biological pathways, regulation

INTRODUCTION

Reproduction is one of the most important traits in livestock production particularly for females. Selection for higher prolificacy in domestic sheep (Ovis aries) has led to variable litter size (LS) within and among breeds. For example, individual litter size of 1 to 8 has been recorded in the Hu sheep and Finnsheep (Yue, 1996; Davis et al., 2006a).
Previous studies reported that the exceptional prolificacy of the Booroola Merino was attributed to a single major gene, while a number of mutations of a major effect on litter size have been identified in other sheep breeds (Table 1; see also Xu and Li, 2017). Vage et al. (2013) detected a mutation FecGr in gene GDF9 strongly associated with litter size in Norwegian White Sheep and Finnish Landrace (Finnsheep) using a genome-wide association analysis. Demars et al. (2013) reported the mutations FecXGr in Grivette sheep and FecXO in Olkuska sheep associated with the highly prolific phenotype by a genome-wide association analysis. Cao et al. (2016) found that nine candidate genes including the well-known FecB mutation played important roles in the variable litter size in Hu and Small-tailed Han sheep through methylated DNA-immunoprecipitation sequencing data. Miao et al. (2016) identified a set of differentially expressed genes (e.g., FecB) between low- and high-prolificacy breeds (Dorset vs. Small-tailed Han sheep) through implementing integrated analysis of miRNAs and lncRNAs. Lassoued et al. (2017) found the mutation FecXBar associated with the prolificacy in Tunisian Barbarine. Despite its great importance the genetic mechanisms of the high prolificacy trait in domestic sheep are still poorly understood, partly due to shortage of studies conducted across multiple prolific sheep breeds. To date, numerous fecundity-associated mutations have been identified in different sheep breeds, but very few mutations have been consistently detected across the breeds. Despite the reproduction of ewes can be affected by the complex interactions of environmental conditions (i.e., climate, density, and food abundance) (Wilson et al., 2009), previous studies suggested that genetic factor could play important roles in the variable litter size of ewes.

In this study, we conducted multiple independent genome-wide association studies (GWAS) on litter size in the sheep breeds of high (Wadi, Hu, Icelandic, Finnsheep, and Romanov) and low (Texel) prolificacy with a litter size ranging from 1 to 6 from different geographic regions (Figure 1A) and genetic origins (Figure 1B) of the world, respectively. Wadi sheep is a high-prolificacy native breed from the Shandong Province of China (Peng et al., 2017). Hu sheep is famous for early sexual maturity and high fecundity, and are distributed in the Taihu Lake area of Eastern China (Yue, 1996). Icelandic and

| Gene       | Mutation | Name, allele symbol | Founder breeds | Reference |
|------------|----------|---------------------|----------------|-----------|
| BMP15      | V299D    | Inverdale, FecXI    | Romney, Inverdale | Galloway et al., 2000 |
|            | Q291Ter  | Hanna, FecXH        | Romney         | Galloway et al., 2000 |
|            | S367I    | Belclare, FecXB     | Belclare       | Hanrahan et al., 2004 |
|            | Q299R    | Galway, FecXG       | Belclare, Cambridge, Small-tailed Han | Hanrahan et al., 2004 |
|            | C321Y    | Lacaune, FecXL      | Lacaune       | Bodin et al., 2007 |
|            | ΔP154S159| Rasa Aragonesa, FecXR | Rasa Aragonesa | Martinez-Royo et al., 2008; Montegaglio et al., 2009 |
| BMPR1B     | Q249R    | Booroola, FecBB     | Booroola Merino, Garole, Javanese, Small-tailed Han, Wadi, Hu | Mulsant et al., 2001; Souza et al., 2001; Wilson et al., 2001; Chu et al., 2011; Zhang et al., 2011; Cao et al., 2016 |
| GDF9       | S395F    | High Fertility, FecGH | Belclare, Cambridge | Hanrahan et al., 2004 |
|            | S427R    | Thoka, FecGT        | Icelandic      | Nicol et al., 2009 |
|            | F345C    | Embarpa, FecGE      | Santa Ines     | Silva et al., 2011 |
|            | V371M    | FecGF               | Norwegian White Sheep, Finnsheep, Landrace, Belclare | Vage et al., 2013; Mullen and Hanrahan, 2014 |
|            | R315C    | Vacaria, FecGV      | Brazilian sheep | de Souza et al., 2012 |
|            | R87H     | FecG1               | Baluchi        | Moradband et al., 2011 |
| B4GALNT2   |          | Lacaune, FecLL      | Lacaune        | Drouilhet et al., 2013 |
|            | Woodlands| Wood-land, FecX2W   | Coopworth      | Davis, 2005 |
| OLKUSKA    |          |                     | Olkuska        | Davis, 2004 |
| BELLE-ILE  |          |                     | Belle-Ile      | Davis, 2005 |
| Unknown    |          |                     |                | Davis et al., 2006b |

TABLE 1 | Genetics variants associated with the fecundity in sheep.
Finnsheep (Finnish Landrace) sheep are northern European high-fecundity breeds (Mullen and Hanrahan, 2014; Eiriksson and Sigurdsson, 2017). Romanov sheep from the Volga Valley shows outstanding reproduction qualities: early sexual maturity, out-of-season breeding and extraordinary prolificacy (Deniskova et al., 2017). The Texel sheep is a relatively low-prolificacy breed originally from the island of Texel in the Netherlands and excels in muscle growth and lean carcasses (Casas et al., 2004). Our results will be important for further genetic improvement of the trait and for better understanding the molecular basis of reproduction in sheep as well as other mammals.

MATERIALS AND METHODS

Sample Collection and Phenotyping

A total of 522 ewes from five sheep breeds of high (Wadi, n = 160; Hu, n = 117; Icelandic, n = 54; Finnsheep, n = 54; and Romanov, n = 78) and one low (Texel, n = 59) prolificacy were collected from farms in China, Iceland, Finland, and Russia (Figure 1A). Animals included were as unrelated as possible based on analysis of pedigree records and farmers’ knowledge. Data for the phenotype of litter size and the total number of litters collected from farm records are shown in Figure 2. The litter size ranged from 1 to 6 based on parity from 1 to 11 in six sheep breeds. Genomic DNA was extracted from the ear marginal tissues following a standard phenol/chloroform method and was diluted to 50 ng/µl for the SNP BeadChip genotyping (Köchl et al., 2005), except for the Icelandic samples which were isolated from whole-blood using MasterPure™ Complete DNA Purification Kit (Epicentre Biotech) following the manufacturers protocol.

Genotyping and Quality Control

All the samples were genotyped using the Ovine Infinium HD BeadChip according to the manufacturer’s protocol. Genotypes of a total of 606,006 SNPs were obtained (genotype and phenotype datasets¹). We implemented quality control of these SNPs using PLINK v1.07 software (Purcell et al., 2007). The SNPs or individuals were excluded if they met any of the criteria: (1) no chromosomal or physical location, (2) call rate < 0.95, (3) missing genotype frequency > 0.05, and/or (4) minor allele frequency (MAF) < 0.05. SNPs were excluded from the analysis if a p-value of Fisher’s exact test for Hardy–Weinberg equilibrium less than 0.001.

Genetic Relationships and Population Structure

To investigate the genetic relationships and population structure among the six domestic sheep, we performed global $F_{ST}$.
neighbor-joining (NJ) tree and principle component analysis (PCA). The global $F_{ST}$ value was calculated using GENEP0P v4.2 (Raymond and Rousset, 1995). The genetic distances between populations were calculated using an identity by state (IBS) similarity matrix (Kang et al., 2010). Then, the distances were used to construct a NJ tree with 1000 bootstraps using the package PHYLIP v.3.695 (Felsenstein, 1989). In addition, PCA was conducted using the SmartPCA program from the EIGENSOFT package version 4.2 (Patterson et al., 2006) based on the genotypes data.

**Genome-Wide Association Analysis**

To explore genetic structure within the breeds, multidimensional scaling (MDS) analysis was performed based on the independent SNPs using PLINK v1.07. Firstly, we implemented the option of 'indep-pairwise 50 5 0.05' in PLINK v1.07, which calculated pairwise linkage disequilibrium (LD) in a 50-SNP-window shifted at a pace of five SNPs. If the LD estimate was $r^2 > 0.05$, one of the pairs of SNPs was removed (Purcell et al., 2007). The independent SNPs retained by the LD criteria were then used in the MDS analysis, and the results were plotted using the GenABEL package in R v3.2.2 (Aulchenko et al., 2007).

We performed genome-wide association studies within five sheep breeds of high prolificacy (Wadi, Hu, Icelandic, Finnsheep, and Romanov) and one low prolificacy (Texel) using the case/control design. We ranked all individuals within the breeds according to their litter size from the highest to lowest. Then, we selected individuals from two tails for each breed as ‘case’ and ‘control,’ respectively. Based on the distribution of phenotypes, 114 samples (LS $\geq$ 2) in Wadi, 66 samples (LS $\geq$ 2) in Hu, 20 samples (LS $\geq$ 2) in Icelandic, 37 samples (LS $\geq$ 2.5) in Finnsheep, 40 samples (LS $\geq$ 2.5) in Romanov and 28 samples (LS $\geq$ 1.6) in Texel sheep were selected as ‘cases,’ while 28 samples (LS $\leq$ 1) in Wadi, 15 samples (LS $= 1$) in Hu, 15 samples (LS $\leq$ 1.75) in Icelandic, 9 samples (LS $\leq$ 2) in Finnsheep, 26 samples (LS $\leq$ 2) in Romanov and 14 samples (LS $\leq$ 1.33) in Texel sheep were selected as ‘controls.’ In the GWAS, we used the function of “qtscore” in the GenABEL package. Associated SNPs were identified at both the genome-wide and chromosome-wise significance levels ($p < 0.05$) after the Bonferroni correction (Bonferroni, 1936). To account for systematic biases caused by within-population substructure, the first and second dimensions from the MDS analyses were used as the covariates (Price et al., 2006). The correlation analysis between litter size and parity within breeds showed that there were significant effects between litter size and parity in four breeds (Wadi, Hu, Icelandic, and Texel), and the effect of parity 1 on litter size was less than that of parities 2 through 10 (Supplementary Table S1 and Supplementary Figure S1). However, the parity of individuals within breeds was different, and we mainly focused on the mean of litter size of individual (total litter size/parity) in per breed. Therefore, we excluded the effect of parity from the model. The Quantile–Quantile (Q–Q) plots were visualized by plotting the distribution of obtained vs. expected genome-wide $p$-values. For genotype effect of potential SNPs on litter size in each breed, differences between means were analyzed by the Student's t-test. The $p < 0.05$ was considered statistically significant. All the results were presented as mean ± standard error (SE). We implemented pairwise tests of linkage disequilibrium (LD) between the most significant SNPs and their flanking SNPs within approximately 1 Mb upstream and downstream using PLINK v1.07. Regional association plots were generated using the R package v3.2.2.

**Bioinformatics Analysis**

We annotated the genes associated with litter size in each breed using the O. aries assembly Oar_v4.0. Further, we submitted the genes to the DAVID (database for annotation, visualization and integrated discovery) database for gene ontology (GO) enrichment and pathways analyses (Huang et al., 2009a,b). The $p$-value of 0.1 and at least two genes from the input gene list in the enriched category were considered for the enriched GO terms. Also, we investigated the protein–protein interaction network for the candidate genes using the STRING database version 10.5 (Szklarczyk et al., 2017). In addition, differential expressions of the candidate genes in various tissues were examined using the EMBL-EBI Expression Atlas database (Petrysak et al., 2016).

**RESULTS**

**Population Relationship and Differentiation**

Pairwise $F_{ST}$ value varied from 0.023 to 0.104 among the populations with the least genetic differentiation observed between Wadi and Hu sheep breeds (Supplementary Table S2). The NJ tree showed that these breeds were clustered into two major groups according to their Chinese and European origins (Figure 1B). A similar geographic pattern was seen in the PCA analyses with the grouping of Wadi and Hu sheep separated from the other four European breeds (Supplementary Figure S2).

**Genome-Wide Association Analysis**

After the quality control, 508,444 SNPs and 114 individuals (91 cases vs. 23 controls) in Wadi, 506,031 SNPs and 80 individuals (66 cases vs. 14 controls) in Hu, 443,125 SNPs and 23 individuals (8 cases vs. 15 controls) in Icelandic, 492,165 SNPs and 37 individuals (28 cases vs. 9 controls) in Finnsheep, 465,794 SNPs and 38 individuals (29 cases vs. 9 controls) in Romanov, 475,955 SNPs and 39 individuals (28 cases vs. 11 controls) in Texel sheep were retained in the working dataset for the GWAS. We did find several animals outlying the clusters of cases, which might cause biases in the association analyses (Supplementary Figure S3). We have repeated the association analyses without these animals, and found the results are very similar. Thus, we did not exclude these animals in the association analyses due to the small sample size for the breeds. The resulting genomic inflation factors were equal to 1.07 in Wadi, 1.14 in Hu, 1.12 in Iceland, 1.14 in Finnsheep, 1.10 in Romanov, and 1.05 in

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2http://www.ncbi.nlm.nih.gov/gxa/

3https://david.ncifcrf.gov/

4https://www.ebi.ac.uk/gxa/home/
Texel sheep, suggesting well-controlled population stratifications (Supplementary Figure S4).

In Wadi sheep, we detected 59 and 8 SNPs at the chromosome-wise and genome-wide ($p < 1.92 \times 10^{-6}$) 5% significance after the Bonferroni correction, respectively (Figure 3A and Supplementary Tables S3, S4). We observed a high level of LD between the top significant SNP rs416717560 and rs421635584 located in gene BMPR1B (Figure 4A). For the SNP rs416717560, average litter size of individuals with the G/G genotype ($n = 115$, LS = 2.05 ± 0.06) was significantly ($p < 0.01$) higher than that of the ewes with the A/G ($n = 15$, LS = 1.47 ± 0.16) genotype (Figure 5A). Also, we found three additional significant
SNPs (rs429416173, rs402803857, and rs160917020) neighboring genes BMPR1B, FBN1, and MMP2 (Table 2 and Supplementary Table S3).

In Hu sheep, we identified 98 and 9 SNPs at the chromosome-wise and genome-wide ($p < 2.18 \times 10^{-6}$) 5% significance after Bonferroni correction (Figure 3B and Supplementary Tables S3, S4). The regional plot showed that the top significant SNPs rs429755189 and rs420460180 on chromosome 17 were in an LD block that contained gene GRIA2 (Figure 4B). For the rs429755189, average litter size of individuals with the genotypes G/G ($n = 38$, LS = 1.99 ± 0.07) and A/G ($n = 52$, LS = 1.94 ± 0.06) were significantly ($p < 0.001$) higher than that of ewes with the genotype A/A ($n = 20$, LS = 1.40 ± 0.09) in the present population (Figure 5B). Among these significant SNPs, 3 (rs406357666, rs427436644 and rs412185353) are located within the genes SMAD1 and CTNNB1 (Table 2 and Supplementary Table S3).

In Icelandic sheep, we found 22 SNPs at the chromosome-wise 5% significance after the Bonferroni correction (Figure 3C and Supplementary Tables S3, S4). The top significant SNP rs429836421 on chromosome 3 was located within gene NCOA1 (Figure 4C). For rs429836421, average litter size of individuals with the A/G genotype ($n = 19$, LS = 2.03 ± 0.05) is significantly
(p < 0.05) higher than that of the ewes with the genotype A/A (n = 33, LS = 1.81 ± 0.04) (Figure 5C).

In Finnsheep, we detected 102 and 6 SNPs at the chromosome-wise and genome-wide (p < 3.64 × 10⁻⁶) 5% significance after the Bonferroni correction, respectively (Figure 3D and Supplementary Tables S3, S4). The regional plot revealed strong LD between the top significant SNP rs412280524 and its neighboring SNPs rs401960737 and rs407751830 harbored gene INHBB (Figure 4D). For the SNP rs412280524, litter size of ewes with the genotype A/A (n = 40, LS = 2.84 ± 0.09) is significantly (p < 0.001) higher than that of the ewes with the genotype A/G (n = 13, LS = 2.08 ± 0.16) (Figure 5D). Also, five additional significant SNPs (rs160509574, rs417444297, rs404890873, rs401746929, and rs402764237) were found to be located near to genes FLT1, NF1, PTGS2, and PLCB3 (Table 2 and Supplementary Table S3).

In Romanov sheep, we identified 77 and 2 SNPs at the chromosome-wise and genome-wide (p < 4.56 × 10⁻⁶) 5% significance after the Bonferroni correction (Figure 3E and Supplementary Tables S3, S4). The top significant SNP rs423810437 on chromosome 7 was in the gene ESR2 (Figure 4E). For rs423810437, litter size of ewes with the genotype A/A (n = 69, LS = 2.50 ± 0.06) is significantly (p < 0.001) higher than that of the ewes with the genotype A/G (n = 8, LS = 1.79 ± 0.18) (Figure 5E).

In Texel sheep, we observed 133 SNPs at the chromosome-wise 5% significance after the Bonferroni correction (Figure 3F and Supplementary Tables S3, S4). The regional plot showed that the top significant SNPs rs161146164 and rs413776054 on chromosome 16 were in a strong LD region containing one functional gene GHR (Figure 4F). For rs161146164, litter size of ewes with the genotype A/A (n = 53, LS = 1.64 ± 0.05)
is significantly \((p < 0.01)\) higher than that of the ewes with the genotype A/C \((n = 6, \text{LS} = 1.15 \pm 0.14)\) (Figure 5F). The two mutations \((rs161146164, \text{Asn} > \text{His}; \text{rs}413776054, \text{Pro} > \text{Ser})\) cause the amino acid change in coding region of the GHR gene. In addition, we found eight additional significant SNPs \((rs42666828, rs409969387, rs410595930, rs401207152, rs413148060, rs405994606, rs161612044, \text{and rs412251543})\) surrounding genes \(\text{ESR1, ETS1, FLI1, SPP1,} \text{and MMP15} (\text{Table 2 and Supplementary Table S3}).\)

In addition to the source breed where the target SNPs have been detected, we further assessed genotype effect of the most significant SNPs on litter size in the five sheep breeds. In general, genotypes of the target SNPs did not show significant association with increased litter size in the breeds other than the source breed (Supplementary Table S7). Nevertheless, we observed some exceptions. For example, the genotype A/G of \(rs429836421\), which was identified in Icelandic sheep, showed significant associations with increased litter size in both Icelandic and Hu sheep breeds. However, a lack of homozygotes for the SNPs such as the genotype G/G for \(rs412280524\) in Finnsheep, G/G for \(rs423810437\) in Romanov and C/C for \(rs161146164\) in Texel sheep could be because of low frequency of the mutations and small sample size.

**Bioinformatics Analysis**

We found significantly \((p < 0.1)\) enriched GO terms associated with reproduction for the candidate genes. The GO clusters were primarily enriched in the categories of ovarian and oocyte development \((\text{PTGS2, BMPR1B, INHBB, CTNNB1, MMP2, MMP15, FBN1, GHR, and SPP1})\), phospholipase C activity \((\text{FLTI} \text{ and ESR1})\), SMAD protein \((\text{INHBB and SMADI})\) and BMP signaling \((\text{SMADI1} \text{ and BMPRI1B})\) and positive regulation of transcription \((\text{NCOA1, FLI1, ESR1, ESR2, CTNNB1, ETS1, and BMPRI1B})\), all of which are involved in the folliculogenesis, follicle growth and granulosa cell proliferation (Figure 6 and Supplementary Table S5). Another relevant GO category was hindbrain development \((\text{SMADI1} \text{ and CTNNB1})\), which participated in regulating ovulation \((\text{Baird et al., 2006})\). In addition, we detected 11 genes \((\text{i.e., PLCB3, ESR1, ESR2,})\)
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FIGURE 6 | Gene ontology (GO) enrichments based on the functional genes surrounding the significant SNPs at the chromosome-wise 5% level.

FIGURE 7 | Protein–protein interaction networks identified by using STRING database. Each line indicated known signaling pathways and protein complexes.

MMP2, NCOA1, CTNNB1, INHBB, SMAD1, BMPR1B, PTGS2, and GRIA2) involved in estrogen, thyroid hormone, TGF-beta, retrograde endocannabinoid and hippo signaling pathways, and these pathways played important roles in regulating follicle growth and ovulation in livestock (Supplementary Table S5). However, we observed different GO terms for the candidate genes in different sheep breeds. For example, I-SMAD binding were enriched in Hu sheep, and chromatin binding were enriched in Texel sheep (Supplementary Table S6). In the gene network analysis, we observed that 16 genes (i.e., BMPR1B, FBN1, MMP2, SMAD1, CTNNB1, GRIA2, NCOA1, FLT1, NF1, PTGS2, PLCB3, ESR2, ESR1, ETS1, SPP1, and GHR) showed protein–protein interactions in the network (Figure 7). Expression data further showed that the genes BMPR1B, FBN1, MMP2, GRIA2, SMAD1, CTNNB1, NCOA1, NF1, FLT1, PTGS2, PLCB3, ESR2, ESR1, GHR, ETS1, MMP15, FLI1, and SPP1 were either highly or moderately expressed in reproduction-related tissues such as ovary, uterine cervix, placenta, corpus luteum, cerebellum, pituitary gland or uterus in sheep (Figure 8). Also, gene INHBB showed a high expression in ovary and uterus of Mus musculus 5.

DISCUSSION

In this study, we conducted multiple independent GWAS in different sheep breeds to investigate the genetic mechanisms underlying the litter size in sheep. Coupled with population relationship and bioinformatics analyses, the GWAS identified different genes associated with the litter size in different breeds and revealed their differentially genetic regulation mechanisms associated with follicle growth and ovulation in the reproduction of ewes.

The diverse biological pathways identified from the novel genes annotation play an important role in follicle growth and ovulation of females in different sheep breeds (Figure 9). The three genes identified in Wadi sheep, BMPR1B, FBN1, and

5https://www.ebi.ac.uk/gxa/home/
MMP2, all play a crucial role in regulating hormone secretion (Mulsant et al., 2001; Basini et al., 2011; Zhang et al., 2011; Zhai et al., 2013). For example, BMPR1B gene can lead to an increased density of the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) receptors with a concurrent reduction in apoptosis to increase the ovulation rate of ewes (Regan et al., 2015; Hu et al., 2016). As the main component of microfibrils in the extracellular matrix, the gene FBN1 regulates cumulus cell apoptosis by reducing the expression level of BMP15 involved in estrogen signaling in porcine ovaries (Zhai et al., 2013). The MMP2 gene plays a key role in ovulation and follicle atresia by regulating FSH and insulin like growth factor 1 (IGF1) (Knapp and Sun, 2017). In Hu sheep, the three genes GRIA2, SMAD1, and CTNNB1 are related to estrogen response element (Chang et al., 2013; Kumar et al., 2016; Vastagh et al., 2016). For example, the gene GRIA2 has been shown to participate in the glutamatergic pathway that regulates gonadotropin-releasing hormone (GnRH), a known prerequisite of the subsequent hormonal cascade inducing the ovulation in mice (Vastagh et al., 2016). The gene SMAD1 encodes an intracellular BMP signaling molecule, which is involved in mediating ovulation rate of ewes (Xu et al., 2010). The CTNNB1 gene enhances FSH and...
LH actions in follicles by stimulating WNT/CTNNB1 pathway and G protein-coupled gonadotropin receptors in female (Fan et al., 2010). In Icelandic sheep, the gene NCOA1 can alter the expression of multiple key genes PBP, AIB3, and FGFR2, which are important for aberrant labyrinth morphogenesis of the placenta and embryonic lethality (Chen et al., 2010; Huang et al., 2011). In Finnsheep, the five candidate genes INHBB, NFI, FLT1, PTGS2, and PLCB3 played important roles in the development of folliculogenesis and LH signaling (Ding et al., 2006; Tal et al., 2014; De Cesaro et al., 2015; Ben Sassi et al., 2016; Cadoret et al., 2017). For example, the INHBB gene encodes an inhibitor of apoptosis, which regulates porcine ovarian follicular atresia (Terenina et al., 2017). The coding region of gene NFI presents non-CpG methylation in the murine oocyte, which plays a critical role in mammalian development (Haines et al., 2001). The FLT1 gene has an important role in the activity of vascular endothelial growth factor that linked to folliculogenesis (Celik-Ozenci et al., 2003). The PTGS2 gene plays a critical role in the ovulation by stimulating LH signaling in zebrafish (Tang et al., 2017). The PLCB3 gene is highly expressed in bovine cells of the ovulatory-sized follicles, with the role of activating LH/LHR signaling (Castilho et al., 2014). In Romanov sheep, the gene ESR2 activates ovulation and regulates preovulatory follicle maturation through regulating estrogen response element (Laliotis et al., 2017; Rumi et al., 2017). In Texel sheep, the six candidate genes ESR1, GHR, ETS1, MMP15, FLI1, and SPP1 are relevant to estrogen and follicular growth (Putnova et al., 2001; Bachelot et al., 2002; Munoz et al., 2007; Xiao et al., 2009; Hatzziroodos et al., 2015; Og iwara and Takahashi, 2017). As a key gene affecting estrogen biosynthesis, ESR1 gene functions similarly to ESR2, and is critical for follicular growth and successful ovulation in ewes (Foroughinia et al., 2017). The GHR gene plays a role in follicular growth through stimulating IGF1 in mice (Bachelot et al., 2002). The ETS1 gene was linked to the regulator of protein signaling protein-2 (RGS2) involved in the ovulation in bovine (Sayasith et al., 2014). As a proteolytic enzyme gene, the MMP15 gene has been shown to mediate LH and its receptor in the preovulatory follicles of teleost medaka (Ogiwara and Takahashi, 2017). The FLI1 gene encodes a critical transcription factor, which regulates gene ETS1 (Vo et al., 2017). The SPP1 gene accounts for establishing and maintaining cellular interactions between steroidogenic and non-steroidogenic cells during the development of corpus luteum (Poole et al., 2013). In addition, the GO categories as well as protein–protein network and expression analysis showed that these genes played an essential role in follicle growth and ovulation of ewes. However, further expression analyses of these genes in each breed are necessary in future study. Taken together, the apparent difference for the litter size among the breeds might be explained by diverse regulation mechanisms.

Also, we calculated genetic differentiation among populations using the global FST, PCA, and NJ tree methods to obtain a refined picture of population genetic relationships. The result showed that the genetic groups were consistent with the geographic origins of the breeds. The different genetic mechanisms associated with physiological processes for the litter size among sheep breeds could be related to the various environments in different geographic regions.

We noticed that previous studies had identified several genes of major effect such as BMP1R, BMP15, and GDF9 for the prolificacy in ewes (Table 1). Different from early investigations, we detected a set of novel genes for the litter size in ewes. The main reason could be that most of early studies are based on genome-wide selection tests between prolific and non-prolific breeds using a lower density of SNPs. Instead, here we implemented GWAS within specific sheep breeds of high or low prolificacy using a high density SNP BeadChip array, which should lead to more reliable associations. In addition, the difference in threshold value used to define the 'case' and 'control' groups for each breed was also another potential influential factor. When we implemented the GWAS using a two-step approach via the general linear model and genome-wide efficient mixed-model analysis (GEMMA), we did not find interesting candidate genes associated with reproduction across the six breeds (see Supplementary Material for further details).

The fact that no candidate genes associated with reproduction were detected could be due to that the power to detect such associations will be weak when treating the trait of interest as quantitative given the small sample size. Also, these populations could have been subjected to selection on litter size through environmental variables such as climate and diet. However, we did not obtain data for local environmental variables in our data. Thus, environmental variables as well as the age of reproduction for the ewes were not taken into account in the model of the GWAS, which would be essential for future study.

CONCLUSION

We revealed a set of novel functional genes for the litter size in different sheep breeds across the world. Our results suggested differentially genetic regulation mechanisms for the functional genes in the reproduction of sheep. The significant SNPs and genes identified here are useful for future molecular-based breeding for a higher fertility. Also, our results provide important insights into the regulation of reproduction in sheep and other mammals.

AUTHOR CONTRIBUTIONS

M-HL conceived and designed the project. FW, Z-QS, Y-LR, MS, EE, JH, JK, and TK collected the samples. X-LX extracted the DNA. JK provided help in Beadchip genotyping. S-SX and LG analyzed the data. S-SX wrote the paper with contributions from M-HL. All authors reviewed and approved the final manuscript.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 91731309 and 31661143014), the Taishan Scholars Program of Shandong
Province (No. ts201511085), the National Transgenic Breeding Project of China (2014ZX0800952B), the Academy of Finland (Grant No. 250633), and the Climate Genomics for Farm Animal Adaptation (ClimGen) Project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2018.00118/full#supplementary-material

REFERENCES

Aulchenko, Y. S., Ripke, S., Isaacs, A., and van Duijn, C. M. (2007). GenABEL: an R library for genome-wide association analysis. Bioinformatics 23, 1294–1296. doi: 10.1093/bioinformatics/btm108

Bachelot, A., Monget, P., Imbert-Bolloire, P., Coshigano, K., Kopchick, J. J., Kelly, P. A., et al. (2002). Growth hormone is required for ovarian follicular growth. Endocrinology 143, 4104–4112. doi: 10.1210/endo-2002-220087

Baird, D. T., Cnatinnguiss, S., Collins, J., Evers, J. L. H., Glasier, A., Heitmann, B. L., et al. (2006). Nutrition and reproduction in women. Hum. Reprod. Update 12, 193–207. doi: 10.1093/humupd/dmi003

Bassini, G., Bussolati, S., Baioni, L., and Grasselli, F. (2011). Gelatinasins (MMP2 and MMP9) in swine antral follicle. Biofactors 37, 117–120. doi: 10.1002/biof.1153

Ben Sassi, N., Gonzalez-Recio, O., de Paz-del Rio, R., Rodriguez-Ramilo, S. T., and Fernandez, A. I. (2016). Associated effects of copy number variants on economically important traits in Spanish Holstein dairy cattle. J. Dairy Sci. 89, 6371–6380. doi: 10.3168/jds.2015-10487

Bodin, L., Di Pasquale, E., Fabre, S., Bontoux, M., Monget, P., Persani, L., et al. (2007). A novel mutation in the bone morphogenic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. Endocrinology 148, 393–400. doi: 10.1210/endo-2006-0764

Bonferroni, C. E. (1936). Teoria statistica delle classi e calcolo delle probabilita’. Pubbl. R Ist. Sup. Sci. Econ. Commer. Fir. 8, 3–62.

Cadoret, V., Frapsauce, C., Jarrier, P., Maillard, V., Bonnet, A., Locatelli, Y., et al. (2017). Molecular evidence that follicle development is accelerated in vitro compared to in vivo. Reproduction 153, 493–508. doi: 10.1530/Rep-16-0627

Cao, J. X., Wei, C. H., Zhang, S. Z., Capellini, T. D., Zhang, L., Zhao, F. P., et al. (2007). GenABEL: an Icelandic, Finnish, Romanov, and Texel sheep. Gray and black rings represent association statistics before and after correction for population stratification, respectively.

Celi–Ozenci, C., Akkoyunlu, G., Kayisli, U. A., Ariç, A., and Demir, R. (2003). Localization of vascular endothelial growth factor in the zona pellucida of developing ovarian follicles in the rat: a possible role in destiny of follicles. Histochem. Cell Biol. 120, 383–390. doi: 10.1007/s00418-003-0586-4

Chang, H. M., Cheng, J. C., Klausen, C., and Leung, P. C. K. (2013). BMP15 suppresses progesterone production by down-regulating STAR via ALK3 in human granulosa cells. Mol. Endocrinol. 27, 2093–2104. doi: 10.1210/me.2013-1233

Chen, X., Liu, Z., and Xu, J. (2010). The cooperative function of nuclear receptor coreactor 1 (NCOA1) and NCOA3 in placental development and embryo survival. Mol. Endocrinol. 24, 1917–1934. doi: 10.1210/me.2010-0201

Chu, M. X., Jia, L. H., Zhang, Y. J., Jin, M., Chen, H. Q., Fang, L., et al. (2011). Polymorphisms of coding region of BMPR-IB gene and their relationship with litter size in sheep. Mol. Biol. Rep. 38, 4071–4076. doi: 10.1007/s11033-010-0526-z

Davis, G. H. (2004). Fecundity genes in sheep. Anim. Reprod. Sci. 82–83, 247–253. doi: 10.1016/j.anireprosci.2004.04.001

Davis, G. H. (2005). Major genes affecting ovulation rate in sheep. Genet. Sel. Evol. 37, S11–S23. doi: 10.1016/j.gene.2004.02.062

Davis, G. H., Balakrishnan, L., Ross, I. K., Wilson, T., Galloway, S. M., Lumsden, B. M., et al. (2006a). Investigation of the Booroola (FecB) and Inverdale (FecX) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Anim. Reprod. Sci. 92, 87–96. doi: 10.1016/j.anireprosci.2005.06.001

Davis, G. H., Farquhar, P. A., O’Connell, A. R., Everett-Hincks, J. M., Wishart, P. J., Galloway, S. M., et al. (2006b). A putative autosomal gene increasing ovulation rate in Romney sheep. Anim. Reprod. Sci. 92, 65–73.

De Cesaro, M. P., Macedo, M. P., Santos, J. T., Rosa, P. R., Ludke, C. A., Rissi, V. B., et al. (2015). Natriuretic peptides stimulate oxytocic meiotic resumption in bovine. Anim. Reprod. Sci. 159, 52–59. doi: 10.1016/j.anireprosci.2015.05.012

Demars, J., Fabre, S., Surly, J., Rossetti, R., Gilbert, H., Persani, L., et al. (2011). Genome-wide association studies identify two novel BMP15 mutations responsible for an atypical hyperprolificacy phenotype in sheep. PLoS Genet. 9:e1003482. doi: 10.1371/journal.pgen.1003482

Deniskova, T., Dotsev, A. V., Selionova, M., Wimmers, K., Reyer, H., Kharzinova, V. R., et al. (2017). Whole-genome single nucleotide polymorphism study of 8251280 of F1 ewes in fall mating seasons. J. Anim. Sci. 82, 1280–1289. doi: 10.2527/anirep.2004.011

Castillo, A. C., Nogueira, M. F., Fontes, P. K., Machado, M. F., Satrapa, R. A., Razza, E. M., et al. (2014). Ovarian superstimulation using FSH combined with equine chorionic gonadotropin (eCG) upregulates mRNA-encoding proteins involved with LH receptor intracellular signaling in granulosa cells from Nelore cows. Theriogenology 82, 1199–1205. doi: 10.1016/j.theriogenology.2014.06.011
Köchl, S., Niederstätter, H., and Parson, W. (2005). DNA extraction and Feary, E. S., Juengel, J. L., Smith, P., French, M. C., O'Connell, A. R., Lawrence, Eiriksson, J. H., and Sigurdsson, A. (2017). Sources of bias, genetic trend and Haines, T. R., Rodenhiser, D. I., and Ainsworth, P. J. (2001). Allele-specific non- Galloway, S. M., McNatty, K. P., Cambridge, L. M., Laitinen, M. P., Juengel, J. L., Hatzirodos, N., Hummitzsch, K., Irving-Rodgers, H. F., and Rodgers, R. J. Huang, H. Y., Li, S. F., Zhao, Z. H., Liang, Z., Zhang, J., and Ding, Y. R. (2011). Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009b). Bioinformatics獲取 sensualis cells from small and large antral ovarian follicles. PLoS One 10:e0119800. doi: 10.1371/journal.pone.0119800 Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009b). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 37, 1–13. doi: 10.1093/nar/gkn923 Huang, H. Y., Li, S. F., Zhao, Z. H., Liang, Z., Zhang, J., and Ding, Y. R. (2011). Association of polymorphisms for nuclear receptor coactivator 1 gene with egg production traits in the maternal line of Shaobo hens. Br. Poult. Sci. 52, 328–332. doi: 10.1080/00071668.2011.577057 Hu, X. J., Pokharel, K., Pejipo, J., Ghanem, N., Zhaboyev, I., Kantanen, J., et al. (2016). Identification and characterization of miRNAs in the ovaries of a highly prolific sheep breed. Anim. Genet. 47, 234–239. doi: 10.1111 age.12385 Kang, H. M., Sul J. H., Service S. K., Zaitlen N. A., Kong S. Y., Freimer, N. B., et al. (2010). Variance component model to account for sample structure in genome-wide association studies. Nat. Genet. 42, 348–354. doi: 10.1038/ng.548 Knapp, E., and Sun, J. J. (2017). Steroid signaling in mature follicles is important for Drosophila ovulation. Proc. Natl. Acad. Sci. U.S.A. 114, 699–704. doi: 10.1073/ pnas.1614338114 Köchli, S., Niederstätter, H., and Parson, W. (2005). DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. Forensic DNA Typing Protoc. 297, 13–30. doi: 10.18311/1-59259-867-6/013 Kumar, M., Camlin, N. J., Holt, J. E., Teixeira, J. M., McLaughlin, E. A., and Tanwar, P. S. (2016). Germ cell specific overactivation of WNT/beta catenin signaling has no effect on folliculogenesis but causes fertility defects due to abnormal foetal development. Sci. Rep. 6:27273. doi: 10.1038/srep27273 Laliotis, G. P., Marantidis, A., and Avdi, M. (2017). Association of BF, RBPs, and ESR2 genotypes with litter size in an autochthonous pig population. Anim. Biotechnol. 28, 138–143. doi: 10.1080/09595986.2016.1242490 Lassoudie, N., Benkhil, Z., Woloszyn, F., Rejez, A., Aouina, M., Rekik, M., et al. (2017). FecX(BAR) a novel BMP15 mutation responsible for prolificacy and female sterility in Tunisian Barbarine sheep. BMC Genet. 18:43. doi: 10.1186/s12863-017-0510-x Martínez-Royo, A., Jurado, J. J., Smulders, J. P., Martí, J. I., Alabart, J. L., Roche, A., et al. (2008). A deletion in the bone morphogenic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep. Anim. Genet. 39, 294–297. doi: 10.1111/j.1365-2052.2008.01707.x Miao, X., Luo, Q., Zhao, H., and Qin, X. (2016). Ovarian transcriptomic study reveals the differential regulation of miRNAs and IncRNAs related to fecundity in different species. Sci. Rep. 6:35299. doi: 10.1038/srep 35299 Montagueo, L. V., Ponz, R., Tejedor, M. T., Lavina, A., and Sierra, I. (2009). A 17 bp deletion in the Bone Morphogenic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. Anim. Reprod. Sci. 110, 139–146. doi: 10.1016/j.anireprosci.2008.01.005 Moradband, F., Rahimi, G., and Gholizadeh, M. (2011). Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-IB with litter size in Iranian Baluchi sheep. Asian Australas. J. Anim. Sci. 24, 1179–1183. doi: 10.5737/ajas.2011.10453 Mullen, M. P., and Hanrahan, J. P. (2014). Direct evidence on the contribution of a missense mutation in GDF9 to variation in ovulation rate of Finnish. PLoS One 9:e95251. doi: 10.1371/journal.pone.0095251 Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., et al. (2001). Mutation in bone morphogenic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proc. Natl. Acad. Sci. U.S.A. 98, 5104–5109. doi: 10.1073/pnas.989157598 Munoz, G., Ovilo, C., Estelle, J., Silio, L., Fernandez, A., and Rodriguez, C. (2007). Association with litter size of new polymorphisms on ESR1 and ESR2 genes in a Chinese European pig line. Genet. Sel. Evol. 39, 195–206. doi: 10.1186/1297- 9686-39-2-195 Nicol, L., Bishop, S. C., Pong-Wong, R., Bendixen, C., Holm, E. L., Bhind, S. M., et al. (2009). Homozygosity for a single base-pair mutation in the oocyte-specific GDF9 gene results in sterility in Thoka sheep. Reproduction 138, 921–933. doi: 10.1530/REP-09-0193 Ogijwara, K., and Takahashi, T. (2017). Involvement of the nuclear progestin receptor in LH-induced expression of membrane type 2-matrix metalloproteinase required for follicle rupture during ovulation in the medaka, Oryzias latipes. Mol. Cell. Endocrinol. 450, 54–63. doi: 10.1016/j.mce.2017.04.016 Patterson, N., Price, A. L., and Reich, D. (2006). Population structure and eigaenanalysis. PLoS Genet. 2:e190. doi: 10.1371/journal.pgen.0020190 Peng, W. F., Xu, S. S., Ren, X., Lv, F. H., Xie, X. L., Zhao, Y. X., et al. (2017). A genome-wide association study reveals candidate genes for the supernumerary nipple phenotype in sheep (Ovis aries). Anim. Genet. 48, 570–579. doi: 10.1111/age.12575 Petryszak, R., Keays, M., Tang, Y. A., Fonseca, N. A. B., Herrera, E., Burdett, T., et al. (2016). Expression Atlas update—an integrated database of gene and protein expression in humans, animals and plants. Nucleic Acids Res. 44, D746–D752. doi: 10.1093/nar/gkv1045 Poole, D. H., Ndiaye, K., and Pate, J. L. (2013). Expression and regulation of secreted phosphoprotein 1 in the bovine corpus luteum and effects on T lymphocyte chemotaxis. Reproduction 146, 527–537. doi: 10.1530/Rep-13- 0190 Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., and Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 38, 904–909. doi: 10.1038/ng1847 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., et al. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. doi: 10.1086/ 519785 Putnova, L., Kolarikova, O., Knoll, A., and Dvorak, J. (2001). Association study of osteopontin (SPPI) and estrogen receptor (ESR) genes with reproduction traits in pigs. Acta Univ. Agric. Sciiv. Mendel. Brun. 49, 69–74.
Raymond, M., and Rousset, F. (1995). Genepop (version-1.2)-population-genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249. doi: 10.1093/oxfordjournals.jhered.a111573

Regan, S. L., McFarlane, J. R., O’Shea, T., Andronicos, N., Arfuso, F., Dharmarajan, A., et al. (2015). Flow cytometric analysis of FSHR, BMPR1B, LHR and apoptosis in granulosa cells and ovulation rate in merino sheep. *Reproduction* 150, 151–163. doi: 10.1530/Rep-14-0581

Rumi, M. A. K., Singh, P., Roby, K. F., Zhao, X., Isqbal, K., Ratri, A., et al. (2017). Defining the role of estrogen receptor beta in the regulation of female fertility. *Endocrinology* 158, 2330–2343. doi: 10.1210/en.2016-1916

Sayasith, K., Sirois, J., and Lussier, J. G. (2014). Expression and regulation of regulator of G-protein signaling protein-2 (RGS2) in equine and bovine follicles prior to ovulation: molecular characterization of RGS2 transactivation in bovine granulosa cells. *Biol. Reprod.* 91, 75–86. doi: 10.1095/biolreprod.114.121186

Silva, B. D. M., Castro, E. A., Souza, C. J. H., Paiva, S. R., Sartori, R., Franco, M. M., et al. (2017). *GDF9* gene is associated with increased ovulation rate and prolificacy in homoygous sheep. *Anim. Genet.* 42, 89–92. doi: 10.1111/j.1365-2052.2010.02078.x

Souza, C. J. H., MacDougal, C., Campbell, B. K., McNeily, A. S., and Baird, D. T. (2001). The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *J. Endocrinol.* 169, R1–R6. doi: 10.1677/joe.0.169R001

Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., et al. (2017). The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic Acids Res.* 45, D362–D368. doi: 10.1093/nar/gkw937

Tal, R., Seifer, D. B., Grazi, R. V., and Malter, H. E. (2014). Folicular fluid placental growth factor is increased in polycystic ovarian syndrome: correlation with ptgs2a expression is required for ovulation in zebrafish. *Adv. Cell. Physiol.* 447, 125–133. doi: 10.1016/j.mce.2017.02.042

Terenina, E., Fabre, S., Bonnet, A., Monniaux, D., Robert-Granier, C., SanCristobal, M., et al. (2017). Differentially expressed genes and gene networks involved in pig ovarian follicular atresia. *Physiol. Genomics* 49, 67–80. doi: 10.1152/physiogenomics.00069.2016

Vage, D. I., Husdal, M., Kent, M. P., Klemetsdal, G., and Boman, I. A. (2013). A missense mutation in growth differentiation factor 9 (GDF9) is strongly associated with litter size in sheep. *BMC Genet.* 14:1. doi: 10.1186/1471-2156-14-1

Vastagh, C., Rodolosse, A., Solyomosi, N., and Liposits, Z. (2016). Altered expression of genes encoding neurotransmitter receptors in GnRH neurons of proestrous mice. *Front. Cell. Neurosci.* 10:230. doi: 10.3389/fncel.2016.00230

Vo, K. K., Jarocha, D. J., Lyde, R. B., Hayes, V., Thom, C. S., Sullivan, S. K., et al. (2017). FLI1 level during megakaryopoiesis affects thrombopoiesis and platelet biology. *Blood* 129, 3486–3494. doi: 10.1182/blood-2017-02-770958

Wilson, A. J., Pemberton, J. M., Pilkington, J. G., Clutton-Brock, T. H., and Kruuk, L. E. (2009). Trading offspring size for number in a variable environment: selection on reproductive investment in female Soay sheep. *J. Anim. Ecol.* 78, 354–364. doi: 10.1111/j.1365-2656.2008.01489.x

Wilson, T., Wu, X. Y., Juengel, J. L., Ross, I. K., Lumsden, J. M., Lord, E. A., et al. (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol. Reprod.* 62, 1225–1235. doi: 10.1095/biolreprod64.4.1225

Xiao, X. H., Liu, A. Q., Wen, H. X., Tian, Y. J., Ni, J., and Liu, G. Y. (2009). Expression and localization of transcription factor Ets-1 in the rat ovary during the estrous cycle and pregnancy. *Fertil. Steril.* 91, 1998–2005. doi: 10.1016/j.fertnstert.2008.02.166

Xu, S.-S., and Li, M.-H. (2017). Recent advances in understanding genetic variants associated with economically important traits in sheep (Ovis aries) revealed by high-throughput screening technologies. *Front. Agr. Sci. Eng.* 4, 279–288. doi: 10.15302/J-FASE-2017151

Xu, Y. F., Li, E. L., Han, Y. D., Chen, L., and Xie, Z. A. (2010). Differential expression of miRNAs encoding BMP/Smad pathway molecules in antral follicles of high- and low-fecundity Hu sheep. *Anim. Reprod. Sci.* 120, 47–55. doi: 10.1016/j.anireprosci.2010.02.009

Yue, G. H. (1996). Reproductive characteristics of Chinese Hu sheep. *Anim. Reprod. Sci.* 44, 223–230. doi: 10.1016/0378-4320(96)01562-X

Zhai, B., Liu, H. Y., Li, X., Dai, L. S., Gao, Y., Li, C. H., et al. (2013). BMP15 prevents cumulus cell apoptosis through CCL2 and FBN1 in porcine oocytes. *Cell. Physiol. Biochem.* 32, 264–278. doi: 10.1159/000354435

Zhang, C. S., Geng, L. Y., Du, L. X., Liu, Z. Z., Fu, Z. X., Feng, M. S., et al. (2011). Polymorphic study of FecX(G), FecG(H) and Fec(B) mutations in four domestic sheep breeds in the Lower Yellow River Valley of China. *J. Anim. Vet. Adv.* 10, 2198–2201. doi: 10.3923/java.2011.2198.2201

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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