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REGULAR ARTICLES

Association of a genetic polymorphism in the BMPR-1B gene, and non-genetic factors with the natural prolificacy of the Colombian-haired sheep

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Abstract

Introduction Colombian-haired sheep (OPC) is a creole breed with very good adaptation to the tropical conditions of our country. In sheep, it has been shown that the litter size (LS) is associated with ovulation rate, the number of fertilized eggs, and embryo survival. Also, LS is determined by genetic and environmental effects. In this sense, the receptor 1B of bone morphogenetic protein (BMPR-1B) has been described as a genetic factor. Therefore, the aim of the present work was to characterize and associate the SNP C864T in the BMPR-1B gene with LS in the specific OPC biotypes Ethiopian and Sudan.

Materials and methods Reproductive history (LS, number of calving in the mother, identification of the father, conception year, and conception period) of 200 OPC sheep was assessed. Additionally, sheep were genotyped by sequencing for the SNP C864T. An association between LS, reproductive history, and C864T variation was performed using a GLM fixed-effect model.

Results The frequency of the T allele (0.75 ± 0.03) was higher than that of the C allele ($P<0.05$). The genotypic frequencies were 0.55 ± 0.06, 0.38 ± 0.04, and 0.07 ± 0.01, for TT, TC, and CC, respectively. An average value of He (0.37 ± 0.03) and HWE ($P=0.97$) was found. The LS found was 1.45 ± 0.15. This varied, between biotypes, with number of calving in the mother, with the father, and at the time of conception ($P<0.05$).

Conclusion The LS varied between genotypes ($P<0.05$). The CC genotype was the most prolific (1.81 ± 0.4), followed by the heterozygous (1.45 ± 0.04) and the TT homozygous (1.09 ± 0.04). However, we did not find a variation between biotypes within the genotypes ($P>0.05$). In conclusion, the polymorphism target in the exon 9 of the BMPR-1B gene and non-genetic factors affected significantly the litter size in the OPC.

Keywords BMPR-1B gene · Litter size · SNP C864T

Introduction

In the Colombian sheep inventory, between 85 and 90% is made up of Colombian-haired sheep (OPC). OPC sheep have three biotypes: Sudan, Ethiopian, and Abyssinian, with some phenotypic differences based on coat color and size (Montes et al. 2013; Flórez et al. 2018), also with some productive variations (Moreno and Grajales 2017; Vergara et al. 2017; Montes et al. 2018). Sheep production is related to grazing, thus increasing the usefulness of agricultural land suitable for cultivation (Moreno and Grajales 2017). In addition, in Colombia, sheep production is carried out in traditional and family systems, frequently located in areas with social problems, low application of biotechnologies, and low budgets for animal production, which are generally related to mixed production systems along with other species such as cattle and goats (Ocampo et al. 2017).

In same way, OPC has been considered a suitable for meat production in grazing systems, since the breed has a good adaptation to the productive conditions of the tropical regions, such as heat tolerance, ectoparasites, and the ability to consume low-value nutritionally pastures (Cuellar-Gamboa et al. 2015; Carrillo and Hernandez 2016; Montes et al. 2019). Other biological advantages of sheep include short generational intervals, high prolificacy, smaller size, and optimal use of various food sources, such as crop residues. Currently, the incorporation of technology into the sheep

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production system comes from different production conditions to the tropics (Moreno and Grajales 2017). Thus, the genetic improvement in the OPC is based on crosses to take advantage of the hybrid vigor (Ocampo et al. 2017; Pineda et al. 2018; Montes et al. 2019) in characteristics associated with growth, but with little effect on the reproductive efficiency of the herd (Simanca et al. 2017).

The most studied indicator of reproductive efficiency in sheep is prolificity or litter size (LS), understood as the average of total born lambs (alive or dead) for each farrowing (Polley et al. 2010; Zuo et al. 2013; Ahlawat et al. 2016; Hernández et al. 2019a). Changes in prolificacy are related to the increased ovulation rate, the number of fertilized eggs, and embryonic survival (Alabart et al. 2016a, b; Pineda et al. 2018). In addition, these changes are determined by genetic and non-genetic effects. The above can include nutrition of the lamb prior to puberty and service, the use of feeding practices such as "flushing," the body condition of the female, the breeding period which is directly linked to the availability of food, the chronological age of the female, or the number of parturition and different hormonal treatments (Vicente-Pérez et al. 2015, Alabart et al. 2016a, b). Among the genetic factors, we can found the race, the effects of inbreeding, and the action of unique genes with a greater effect (Vera et al. 2018). These last, and are recognized as fertility genes (*Fec*) (Luna and Alonso 2014). The three most studied *Fec* are growth differentiation factor 9 (*GDF9*) of which five polymorphisms are known called $FecG^{I}$, $FecG^{E}$, $FecG^{H}$, *FecGT*, and $FecG^{WNS}$, bone morphogenetic protein 15 (*BMP15*) with eight described genetic variants ($FecX^{G}$, $FecX^{H}$, $FecX^{I}$, $FecX^{GR}$, $FecX^{L}$, $FecX^{0}$, $FecX^{B}$, and $FecX^{R}$), and bone morphogenetic protein receptor 1B (*BMPR-1B*) of which only the known phenotype Boorola (*FecB*) so far (Albarella et al. 2015). The *Fec* genes encode low molecular weight proteins of regulatory function, both in the development of the ovary and in the ovulation process (Ahlawat et al. 2016).

Bone morphogenetic protein (*BMP*) receptors are a family of transmembrane serine/threonine kinases; these include *BMPR-1A*, *BMPR-1B*, and *BMPR-2* type receptors. The main function of these genes is participation in the *BMP* signaling pathway, which regulates reproductive processes in various species (Lochab and Extavour 2017). Actually, the *BMPR-2* gene is expressed in granulosa cells as a crucial receptor for *BMP15* and *GDF9*, which have an important role in follicle development in preparation for ovulation (Andreas et al. 2016). However, *BMPR-2* cannot bind to *BMP15* and *GDF9* (Liu et al. 2014; El-Halawany et al. 2018) without the help of the *BMPR-1A* and *BMPR-1B* genes (Rajesh et al. 2018; Tang et al. 2018). The *BMPR-1A* complex, *BMPR-1B*, and *BMPR-2* phosphorylate the *SMAD* receptor of which *SMAD* 1, 5, and 8 are specific for the *BMP* pathway, they then bind to *SMAD4*, and this complex binds to DNA to regulate the transcription of ovulation-related genes (Islam et al. 2019).

The effects of the $FecX^{R}$, $FecG^{H}$, $FecG^{I}$, and Fec^{B} genetic polymorphisms (Pineda et al. 2018; Hernández et al. 2019a, b) associated with prolificacy have been studied in the OPC. However, the possible effects of the *BMPR-1B* gene are unknown. Therefore, the objective of this work was to characterize and associate a single nucleotide polymorphism with natural prolificity in Ethiopian and Sudan OPC biotypes.

Materials and methods

Population, sample collection, and DNA extraction

The reproductive history of 200 females of the OPC breed, belonging to the Ethiopian (n = 100) and Sudan (n = 100) biotypes, was selected from a herd located in the state of Córdoba, Colombia (8° 34′ 38.3″ N, 75° 53′ 45.3″ W)—tropical dry forest, temperature above 27 °C, and 1200mm of precipitation and a relative humidity of 84%—OPC sheep were kept under grazing conditions in *Bothriochloa pertusa* (dry matter: 22.8%; crude protein: 6.6%; FDN: 68.9%; metabolic energy: 1.7 Mcal/kg/DM) and *Brachiaria brizantha* (dry matter: 24.3%; crude protein: 12.1%; FDN: 61.9%; metabolic energy: 1.9 Mcal/kg/DM) meadows with water and salt available at will all the time. All process such as sample collection, handling, and conservation were approved by the Institutional Committee of Bioethics from the University of Sucre, and those were conducted with the ethical, technical, scientific, and administrative standards for research on animals contained in Law 84 by the National Congress of Colombia 1989. Samples were obtained by venipuncture using a 5-mL syringe and anticoagulant (EDTA 7.2 mg) test tubes. Samples were stored at 4 °C until transport to the Animal Reproduction and Genetic Improvement laboratory at the University of Sucre. DNA extraction was performed using QIAGEN's commercial QIAamp® DNA Mini Kit. DNA quantity and quality were evaluated using NanoDrop 2000™ (Thermo Fisher Scientific).

Amplification, sequencing, and genotyping of the BMPR-1B locus

A 304-bp fragment corresponding to exon 9 of the BMPR-1B gene was amplified by conventional PCR, using primers F: 5′-TCTTGGGCTTCATTGCTGCCGAT-3 ′ and R: 5′-TAAA CTTAACAGCCAAGCCCAGGTC-3 ′ (Jia et al. 2019). The PCR reactions were carried out in a final volume of 25 μL containing 10 ng of DNA, 250 nM of each primer, and 1X of the MangoMix™ super mix (Bioline ©). Amplification conditions included initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, ending at 72 °C for 10 min. Amplifications were performed in an Eppendorf® MasterCycler Nexus Gradient

thermocycler. Visualization of the amplified fragments was performed in 2% agarose electrophoresis stained with GelRed™ (Biotium).

Amplified products were purified using Thermo Scientific™ GeneJET™ PCR Purification Kit, according to the manufacturer's instructions. Amplicons were bidirectionally sequenced in MACROGEN USA. The sequences obtained were edited and aligned on the BLAST website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and using the MEGA program see 7.0 (Kumar et al. 2016). The genotyped polymorphism consists of a T by C transition at position 864 of the BMPR-1B gene (Jia et al. 2019).

Statistical analysis

Allelic and genotypic frequencies, observed (Ho) and expected (He) heterozygosity, fixation index (F), and deviations from Hardy-Weinberg equilibrium (HWE) were calculated for each biotype and in the total breed (OPC). These frequencies were compared between biotypes using the Fisher test with a significance of 0.05. All the analyses were performed with the software Arlequin version 3.5.2.2 (Excoffier and Lischer 2010).

From the reproductive records of the herd, the litter size (lambs/female/farrowing) was calculated in 450 births (Ethiopian n = 216 and Sudan n = 234). In addition, some parameters were recognized as non-genetic effects, such as the number of calving of the mother (), the identification of the father (), the time of conception (dry-rain), and the year of conception (2014–2018) for each biotype. The above data were analyzed using descriptive statistics. The prolificity observed in each biotype and for the OPC was associated with the BMPR-1B genotypes found using the GLM fixed-effect procedure, using the software R® version 3.61 according to the following model:

$$Y_{ijklmn} = \mu + A_i + B_j + C_K + D_l + F_n + H_m \cdot \varepsilon_{ijklmn}$$

where:

Y_{ijklmn}	observed prolificity
μ	effect of population mean
A_i	effect of the ith genotype TT, TC, and TC
B_j	effect of the jth birth of the mother j = 1 to 5
C_k	effect of the kth father k = 1 to 6
D_l	effect of the lth year of conception l = 2015 to 2019
F_n	effect of the nth conception period n = dry (December to April) and rain (May to November)
H_m	effect of the mth Ethiopian or Sudan biotype
ε_{ijklnm}	effect of random error

The effect of the father refers to the effect of the male used to mate each female. In addition, in tropical countries, the reproduction systems in sheep are continuous; therefore, the conception period is determined by the weather season, divided into two periods, the rainy period (May to November) and the drought period (December to April).

Table 1. Allelic and genotypic frequencies for the BMPR-1B locus at the OPC breed.

Biotype	Allelic Frequencies		Genotypic Frequencies		
	T	C	TT	CT	CC
Ethiopian	0.77[a]	0.23[a]	0.59[a]	0.35[a]	0.06[a]
Sudan	0.72[b]	0.28[b]	0.51[b]	0.41[b]	0.08[b]
OPC	0.75 ±0.03	0.25 ±0.03	0.55 ±0.06	0.38 ±0.04	0.07 ±0.01

[a, b] Different letters in the same column differ statistically (P< 0.05). Data are expressed as Means ± Standard Deviation.

Results

Genetic diversity at the BMPR-1B locus

The frequency of the T allele (0.75 ± 0.03) was significantly (P<0.05) higher than that to the C allele (0.25 ± 0.03) for the breed OPC in general. In addition, we found significant differences (P<0.05) in the allele frequencies in the Ethiopian and Sudan biotypes with a higher value for the T allele in Ethiopian and the C allele in Sudan (Table 1). The genotype frequencies found in the OPC breed also varied significantly between biotypes (P<0.05). The homozygous TT and CC genotypes presented the highest and the lowest genotypic frequency, respectively (Table 1).

The genetic diversity indices for the locus studied for the entire OPC breed and for biotypes are presented in Table 2. Statistical differences (P<0.05) were found between biotypes for the Ho and He values, with greater than the value in the Sudan biotype to the Ethiopian biotype. The fixation index (F) and the deviations from the theoretical HWE proportions were not significant (P>0.05).

Table 2. Genetic diversity indices for the *BMPR-1B locus* at the OPC breed.

Biotype	Ho	He	F	HWE (*p-valor*)
Ethiopian	0.35[a]	0.35[a]	0.01[a]	0.99
Sudan	0.41[b]	0.40[b]	-0.03[a]	0.98
OPC	0.38 ±0.04	0.37 ±0.03	-0.03	0.97

[a, b] Different letters in the same column differ statistically (P< 0.05). Data are expressed as Means ± Standard Deviation (SD)

Table 3. Reproductive parameters in the OPC breed.

Variable	Mean ± SD	Min	Max	CV
Prolificity	1.45±0.15	1	2	10.31%
Mother parturition	2.50±0.51	1	4	20.11%
Father	90±16.81	40	140	18.61%
Conception period	75±4.61	61	89	6.11%
Conception year	85±11	52	118	12.91%

Data are expressed as Means ± Standard Deviation (SD)

Abbreviations: Min = minimum, Max = maximum, CV = coefficient of variation

Association of the BMPR-1B locus with prolificity in the OPC breed

In general, the prolificacy average was 1.45±0.15 (Table 3). However, the average per sheep was 2.5±0.5 parturitions. In addition, most of the sheep had just one calving (41.75%). Further, 22.95% of sheep had two calving, 14.25% three calving, and 12.25% four calving. Sheep were mated with five males. On average, each ram was responsible for 90 ± 16.8 calving. For each period, an average of 75 ± 4.6 sheep was mated, in a time of 5 years, with a mean of 85 ± 11 pregnancies/year.

The LS varied between biotypes ($P<0.05$) with higher value in Ethiopian. When litter size was assessed, we found that litter size increased significantly at the same time with the number of delivery ($P<0.05$). Therefore, the litter size in the first parturition was 1.43 ± 0.36 lambs in comparison with the fourth parturition where the littler size was 1.46 ± 0.26. On the other hand, we found that the prolificity depended on the father significantly ($P<0.05$), where the maximum value found was 1.50 ± 0.30 and the lowest was 1.42 ± 0.32. About the rainy period, prolificacy was 6.6% higher significantly than in the dry period (P<0.05). Also, the prolificacy varied significantly with the years ($P<0.05$). The data are summarized in Table 4.

Prolificity varied significantly between genotypes ($P<0.05$). The CC genotype was the most prolific, followed by the heterozygous and the TT homozygous. However, we did not find a variation between biotypes within the genotypes ($P>0.05$). The association of prolificacy and genotype for the BMPR-1B locus in the OPC breed is shown in Table 5.

Discussion

We found how the litter size has a major impact on reproductive efficiency in sheep. In this sense, we could postulate that the prolificity could be directly associated with the ovulation rate, which is the result of the variation in the sensitivity of gonadotropin release and to the feedback effects of gonadal steroids (Miao et al. 2018). The above could be affected by both genetic (*BMPR-1B*) and non-genetic factors (Pineda et al. 2018). In this research, the prolificacy mean found (1.45 ± 0.15) was higher than that reported by other authors for this same racial group (Cuellar-Gamboa et al. 2015; Pineda et al. 2018; Hernández et al. 2019b).

In this work, we found that prolificity varied significantly between genotypes to the *BMPR-1B locus* in the OPC breed.

Table 4. Association of biotype effects, number of calving in the mother, identification of the father, conception period, and conception year with prolificity in the OPC breed.

Biotype	**Prolificity**
Ethiopian	1.48 ± 0.36^{a}
Sudan	1.43 ± 0.36^{b}
Number of calving in the mother	**Prolificity**
1	1.43 ± 0.36^{b}
2	1.45 ± 0.25^{ab}
3	$1.46 \pm 0,25^{a}$
4	1.46 ± 0.26^{a}
Identification of the father	**Prolificity**
1	1.50 ± 0.30^{a}
2	1.42 ± 0.32^{c}
3	1.47 ± 0.24^{b}
4	1.43 ± 0.29^{c}
5	1.46 ± 0.28^{b}
Conception period	**Prolificity**
Dry	1.40 ± 0.29^{b}
Rainy	1.50 ± 0.30^{a}
Conception year	**Prolificity**
2015	1.41 ± 0.23^{c}
2016	1.47 ± 0.25^{a}
2017	1.45 ± 0.28^{b}
2018	1.44 ± 0.32^{b}
2019	1.47 ± 0.36^{a}

[a, b,c] Different letters in the same column differ statistically (P< 0.05). Data are expressed as Means ± Standard Deviation (SD)

Table 5. Average genotype prolificity for the BMPR-1B locus in OPC breed biotypes.

Biotype	**Genotypes**		
	TT	**CT**	**CC**
Etiophian	1.12 ± 0.41^{c}	1.48 ± 0.61^{b}	1.84 ± 0.51^{a}
Sudan	1.07 ± 0.51^{c}	1.43 ± 0.41^{b}	1.79 ± 0.41^{a}
OPC	1.09 ± 0.04	1.45 ± 0.04	1.81 ± 0.42

[a, b,c] Different letters in the same column differ statistically (P< 0.05). Data are expressed as Means ± Standard Deviation (SD)

Genetic factor *BMPR-1B* is a member of the *TGF-β* superfamily, and plays a key role in regulating follicular development and ovulation rate, which largely defines litter size (Jia et al. 2019). The first researches on the relationship of the *BMPR-1B* gene and prolificacy identified a polymorphism called Boorola or *FecB* (A746G), which causes loss of function in the protein that promotes steroid production, the ovulation rate in Australian Merino Sheep (Shokrollahi and Morammazi 2018), with additive effects for each copy of the gene (Maskur et al. 2016). Thus, the *FecB* polymorphism has been used as a major gene for prolificacy and has been reported in some breeds around the world, including OPC breed (Hernández et al. 2019b). In addition, Jia et al. 2019 reported the first association of the polymorphism assessed in our research (T864C) with litter size in Dorset breed sheep. Although this mutation is not synonymous, we propose that the *BMPR-1B* exon-9 mutation could be competitively combined with miR-204 to regulate the expression level of *SMADS* protein phosphorylation (Zuo et al. 2013). The T864C mutation in the *BMPR-1B* gene changed the UCA codon for IDU and UCG; the latter is considered a rare codon, which could influence numerous stages of protein metabolism. UCG appears to influence the translation speed, and also could affect protein folding (Jia et al. 2019). Thus, this synonymous mutation could modify the splicing site with the subsequent modification in the mRNA and changes in the specific expressions of the gene, in follicular oocyte cells and granulosa cells (Jia et al. 2019).

On the other hand, similar research conducted in Dorset sheep with a CC genotype had 30% more prolificacy than the average of the other genotypes (Jia et al. 2019). Also, in the research conducted in Mongolian and Small Tail Han breeds, no association was found, but a tendency to be higher in sheep carrying the C allele (Jia et al. 2019). According to the above, the C allele-bearing genotypes could be of zootechnical interest. Regarding allele frequencies, the T allele was the most frequent in Dorset, Mongolian, and Small Tail Han sheep (Jia et al. 2019), which agrees with our work.

The economic effects of the T864C polymorphism in the BMPR-1B gene have not yet been determined; however, an additional gain of € 40/lamb has been estimated in prolific ewes (Legarra et al. 2007; Vadhana et al. 2019). The above could represent an additional gain of € 28.8/lamb and € 14.4/lamb in ewes with CC genotype, with respect to genotypes TT and CT. For other genes related to litter size, the FecXR gene has been reported that having 5% of animals with heterozygous genotype increases income by € 40.85 (P <0.05) compared to herds that do not select for prolificacy (Alabart et al. 2016a, b). On the other hand, in sheep carrying the FecB gene, a 60% higher average profitability has been reported, compared to non-carriers (Gootwine et al. 2001; Ahlawat et al. 2016).

The Sudan biotype had a higher frequency of the genotype of interest (CC) compared to the Ethiopian biotype (P<0.05), but the prolificity observed was greater in the Ethiopian biotype (P<0.05). However, this research is the first work where an association between prolificacy with a fertility gene (*Fec*) was conducted, and it was finding results that contrast with other researches (Pineda et al. 2018; Hernández et al. 2019b).

Regarding the factors that are classified as non-genetic (number of calving in the mother, father, conception time, and conception year), we found that all could affect the litter size. Above results contrast with that reported by Hernández et al. 2019b and Pineda et al. 2018 who showed a non-effect of the number of calving in the mother, father, period, and year of conception on prolificity

Besides, prolificity changed with the number of calving in the mother (Liandris et al. 2012), an indirect measure of the sheep's age and weight (Mohammadabadi and Sattayimokhtari 2013) at the time of mating. The above could be associated with the relationship between maturation and acquisition of an efficiency reproduction, since as the sheep matures and reaches its body and physiological development. It makes them more efficient at pregnancy maintaining, better milk production, and the expression of their maternal ability (Liandris et al. 2012). Under a traditional production system, a sheep in the growth process needs to compete with gestation and reproductive processes to obtain circulating nutrients in the blood, which could reduce and delay their reproductive parameters. In addition, in tropical areas, the mating programs are continuous, and the sheep are generally mated between 20 and 26 kg because of the extensive production model (Magaña-Monforte et al. 2013).

We found that the father effect affected the prolificity of the OPC sheep could be explained by body condition, nutritional status, health status, the rams:sheep ratio in the mating lots, and ram's reproductive resting periods between mating periods, which could affect sperm quality and other reproductive functions such as libido (SanCristobal-Gaudy et al. 2001).

The two variables related to conception date in the sheep had a significant relationship with prolificacy. The high prolificacy in the rainy period is explained by the availability of food, reported by Hinojosa-Cuéllar et al. 2013 in Pelibuey x Blackbelly and its crossings with Dorper and Katahdin in Mexico. In addition, the year effect could be associated with the intrinsic management of the herd; however, we could not conclude about this variable, because data were taken from animal records (SanCristobal-Gaudy et al. 2001).

In conclusion, our results showed that LS in OPC sheep is influenced by non-genetic factors, the SNP C864T located in exon 9 of the BMPR-1B gene and conditions associated with the animal management in the production system. In addition, the knowledge about the C864T gene can be used in the future, for the selection process into the genetic improvement program in order to increase the natural prolificity in the breed.

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Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

Conflicts of interests The authors declare no competing interests.

Ethics approval All process such as sample collection, handling, and conservation were approved by the Institutional Committee of Bioethics from the University of Sucre

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