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Association of the Polymorphisms FecX^{R}, FecG^{H}, and FecG^{I} and Non-Genetic Factors that Affect the Prolificacy of Colombian Creole Sheep

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Abstract

Objective: To determine the effect of the FecX^{R}, FecG^{H} and FecG^{I} genetic polymorphisms and other non-genetic effects on the natural prolificacy of Colombian creole hair sheep. **Materials**: The birth number of the mother, the date of service (season and year), the male used and the prolificacy of 50 OPC females were recorded. The FecX^{R}, FecG^{I} and FecG^{H} loci were genotyped with direct PCR and PCR-RFLP. The allelic and genotypic frequencies, the observed (Ho) and expected heterozygosity (He), the fixation index and the deviations from the Hardy-Weinberg equilibrium (EHW) were calculated. The polymorphisms and non-genetic factors were associated with prolificacy using the GLM procedure. **Findings:** The FecX^{R} and FecG^{H} loci were monomorphic without the mutated allele, which is why the association analysis with prolificacy was not carried out. The FecG^{I} locus of the GDF9 gene was polymorphic with frequencies of G = 0.89 and A = 0.11. The homozygous AA genotype was not found, and the GG and GA genotypes had frequencies of 0.78 and 0.22, respectively. The Ho was greater than the He; this excess of heterozygotes was not significant and neither were the deviations of the EHW ($p>0.05$). The average prolificacy was 1.30 ± 0.30. The genotype in the FecG^{I} locus did not significantly affect the prolificacy ($p = 0.087$) but did so more so in the heterozygotes. The non-genetic effects analyzed, such as the number of the mother's birth, the season and year of conception, and the father, did not affect the natural prolificacy of the OPC ($p> 0.05$). **Application:** The FecG^{I} and FecG^{H} loci were monomorphic, and FecG^{I} locus was polymorphic; however, the genetic variation and non-genetic factors were not associated with the prolificacy.

Keywords: Bone Morphogenetic Protein-15 Gene, Growth Differentiation Factor-9 Gene, Reproduction

1. Introduction

Sheep brought during the conquest of the Americas provided the racial foundation for what is now known as the creole sheep[1]. In Colombia, there are two types of sheep: creole wool (CRL) and Colombian hair sheep (OPC)[2]. In Colombia, sheep production is considered a secondary livestock activity and is carried out in traditional and/or family systems with low input requirements and in mixed production systems with other species[3].

CRL and OPC sheep have important adaptive characteristics to the tropical climate, such as heat tolerance, ectoparasites and the ability to consume grasses with low nutritional value[4]. In spite of this, these production systems have problems with nutrition, reproduction, health, unorganized genetic improvement plans, inadequate facilities and poor business vision, which are reflected in the low production efficiency[5].

To increase the competitiveness of this sector, it is necessary to incorporate new technologies and/or management practices that generate competitive advantages with knowledgeable management systems that adapt to the conditions of primary production in developing countries such as Colombia[3,6].

The adoption of technologies from places with different conditions than those of the Colombian tropics

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presents obstacles to achieving competitiveness, so it is necessary to deepen the different aspects associated with production, thereby improving the productive indexes and the reproductive efficiency[3,6,7]. The reproductive efficiency of a herd can be measured with several parameters, including fertility (number of pregnant or lambing ewes over the number of mated ewes), prolificacy (number of lambs born per ewe lambing) and the survival of the lambs (number of weaned lambs over the number of born lambs)[8]. Of these three parameters, prolificacy has been studied the most. An increase in prolificacy is mainly due to an increase in the ovulation rate, number of fertilized ovules and embryonic survival, which cause an increase in the number of double and triple births[9].

The ovulatory rate is determined by genetic and non-genetic effects. This factor may include the nutrition of the lamb prior to puberty and service, the use of dietary practices such as "flushing", the body condition of the female[8], the season or year of the service, which is directly linked to the availability of food[10], the chronological age of the female or the number of calving and different hormonal treatments[11].

Genetic factors include the breed, the effects of consanguinity and the action of unique genes with a greater effect[8]. These are called fertility genes (Fec)[12]. One of these is located on the X chromosome (FecX); the gene that occupies this locus is the bone morphogenic protein 15 gene (Bone Morphogenetic Protein 15 - BMP-15), of which six phenotypes are known as Inverdale (FecX^{I}), Hanna (FecX^{H}), Belclare (FecX^{B}), Galway (FecX^{G}), Lacaune (FecX^{L}) and Roa (FecX$_{R}$)[11]. The FecX^{R} allele was described in the Aragonese ovine breed; this variant consists of a deletion of 17 base pairs (c.525_541delTGGG TCCAGAAAAGCCC), which introduces a stop codon[13]. FecX^{R} increases the ovulation rate by +0.63 in heterozygous animals, which increases the prolificacy by 0.35 lambs/calving (1.69 +/FecX^{R} vs. 1.34 +/+, p<0.0001); in addition, the homozygous FecX^{R}/ FecX^{R} sheep are sterile[11].

Another related gene is GDF9 (Growth Differentiation Factor 9), located on chromosome 5; this locus is also called FecG and is only expressed in the ovary, essential for folliculogenesis. Three phenotypes are known: High fertility (FecG^{H}), Embrapa (FecG^{E}) and G1 (FecG^{I})[14]. The FecG^{H} polymorphism is a mutation of C1184T, which results in the change of Serine by Phenylalanine in position 77 of the mature peptide. This polymorphism changes the mode of interaction of the receptor type 1 domain of protein[15]; thus, the homozygous genotype TT does not ovulate, whereas, in heterozygotes (CT), the average ovulation rate is +2, as compared to the wild type[16,17]. The FecG^{I} (G/A) polymorphism causes a change of Arginine to Histidine at position 87 of exon 1. The chemical nature of these amino acids suggests that they do not affect the activity of the mature protein[14].

For OPC, the genetic and environmental factors that affect the prolificacy of the breed have not been studied, so the objective of this research was to determine the effect of the genetic polymorphisms FecX^{R}, FecG^{H} and FecG^{I} and other non-genetic effects on the natural prolificacy of Colombian creole hair sheep.

2. Materials and Methods

2.1 Animals, Productive Data and DNA Extraction

The date of service (season and year), the male used and the prolificacy of 50 clinically healthy OPC females from a herd located in the Department of Cordoba, Colombia (9° 10'42.4 "N, 75° 33'51.3" W) were recorded, for a total of 150 events. Likewise, peripheral blood was collected in tubes with an anticoagulant (EDTA 7.2 mg), from which DNA was extracted using the QI Aamp® DNA Mini Kit from QIAGEN. The quantity and quality of the DNA was evaluated using Nano Drop 2000TM (Thermo Fisher Scientific).

2.2 Amplification and Genotyping of the FecX^{R}, FecG^{H} and FecG^{I} loci

The FecX^{R} locus was genotyped with direct PCR using the primer 5'-CTCTGAGACCAAACCGGGTA-3' and 5'TTTGAGGAGCCTCTTCCTGA 3[18]. The FecG^{H} and FecG^{I} loci were genotyped with PCR RFLP, using the primers 5' - CTTTAGTCAGCTGAAGTGGGACAAC 3', R 5'-ATGGATGATGTTCTGCACCATGGTGTGAACCT GA 3' and the enzyme DdeI for the former, and the primers 5' - GAAGACTGGTATGGGGAAATG-3', 5'-CCAATCTGCTCCTACACACCT-3' and the enzyme HhaI for the latter[18]. All PCR reactions were carried out with a final volume of 12.5 µl, containing 20 ng of DNA, 250 nM of each primer and 1X of the super mix MangoMix™ (Bioline ©). The amplification profile of the three polymorphisms included an initial denaturation of 95°C for 5 minutes, followed by 30 cycles of denaturation

at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension of 72°C for 5 minutes. The reactions were carried out in a Master Cycler Nexus Gradient thermo cycler from Eppendorf®. The RFLP was performed with a final volume of 12.5 µl, containing 3 µl of PCR product, 1X of Buffer Tango and 10U of each enzyme; the digestion mix was incubated for 3 hours at 37°C and 20 minutes at 80°C. The PCR product from the $FecX^R$ locus and the restriction products from the $FecG^I$ and $FecG^H$ loci were mixed at a 1:4 ratio with GelRed (Biotium, Inc. USA) and subjected to vertical electrophoresis in 37:1 polyacrylamide gels (acrylamide: bisacrylamide) at 12% at 160 volts for 45 minutes and observed under ultra violet light. The genotypes were assigned as determined by[14,19].

2.3 Analysis of Molecular Data

The genotypic and allelic frequencies, the observed (Ho) and expected heterozygosity (He), the F index and the Hardy-Weinberg equilibrium (EHW) deviations were calculated using the GENALEX program, version 6.520, for the three genetic polymorphisms.

2.4 Analysis of Phenotypic Data

An analysis of the descriptive statistics was carried out for the variables of prolificacy (lambs/female/parturition), season (rainy from May to November, dry from December to April) and year (2012 to 2016) of conception, and number of parturitions for the mothers and fathers. An analysis of variance was performed using the fixed-effect GLM procedure to determine which genetic factors (genetic polymorphisms) and non-genetic factors affected prolificacy, using the R® software (R Development Core Team, 2008) according to the following model:

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

Where:

Y_{ijklmn} = observed prolificacy

m = Effect of the population mean

A_i = Effect of the i-th genotype *o*n the *loci* $FecX^R$, $FecG^H$ and $FecG^I$

B_j = Effect of the j-th delivery of the mother j = 1 to 6

C_k = Effect of the k-th father k = 1 to 6

D_l = Effect of the l-th year of conception l = 2012 to 2016

F_n = Effect of the n-th conception season n = dry (December to April) or rainy (May to November)

e_{ijklmn} = Effect of random error

3. Results and Discussion

3.1 Genetic Polymorphisms

The $FecX^R$ and $FecG^H$ loci were monomorphic (Table 1), and only the wild genotypes were found, so the mutated alleles of each polymorphism were not found ($FecX^R$ and T, respectively). The $FecX^R$ allele has only been found in the Aragonese breed; it has been shown that the $FecX^R$ allele increases the ovulation rate by +0.63 ovules/sheep in heterozygous animals, which causes an increase in the prolificacy of 0.35 lambs/parturition[11]. This polymorphism does not significantly affect the birth weight of offspring, their daily weight gains or the quality of the meat[9,20,21]. In other mutations in the FecX locus, in the Pelibuey breed, a higher proportion of double and triple births were found in heterozygous individuals in the $FecX^G$ and $FecX^L$[22] polymorphisms.

Table 1. Genotypic and allelic frequencies for the three studied loci in the OPC breed

Locus	Genotypic Frequencies			Allele frequencies	
$FecX^R$	**+/+**	**+/$FecX^R$**	**$FecX^R$/ $FecX^R$**	**+**	**$FecX^R$**
	1.00	0.00	0.00	1.00	0.00
$FecG^I$	**GG**	**GA**	**AA**	**G**	**A**
	0.78	0.22	0.00	0.89	0.11
$FecG^H$	**CC**	**CT**	**TT**	**C**	**T**
	1.00	0.00	0.00	1.00	0.00

The absence of the mutated $FecG^H$ allele agrees with the findings[19] for the Araucanian breed of Chile[23] for the Arab and Kordi breeds and[24] in the North African breeds Barbarine, Queue Fine de L'Ouest, Noire de Thibar, Sicilo-Sarde and D'man. On the other hand[25], for the Chios breed, reported frequencies of 65, 11 and 24% for the genotypes CC, CT and TT, respectively, and, for the Karagouniki breed, 76, 24 and 0% for the CC genotypes, CT and TT, respectively.

The absence of the $FecX^R$ and $FecG^H$ alleles in the OPC can be attributed to genetic drift effects that made

it impossible to sample animals with any of the mutations to which these polymorphisms are fixed in the breeds, in which FecX^{R} was reported in the Aragonese breed[13] and FecG^{H} in the Belclare and Cambridge breeds[16], and the fact that these breeds did not participate in OPC training because of a lack of reproductive management and genetic improvement plans for OPC.

The FecG^{I} locus was polymorphic (Table 1), with a higher frequency of the wild genotype (GG) and an absence of the mutated genotype (AA). The frequency of allele A at this locus was lower than that of allele G. The only analysis report of this polymorphism in creole breeds was presented by[19] for the Araucana breed of Chile, with a frequency of 22%. In the Corriedale breed, the reported frequency is 27%[26]. Higher frequencies of this allele were reported for the Afshari, Baluchi, Makui and Merhaban breeds by[27]. Lower frequencies were reported by[25] for the Karagouniki breed and by[23] for the Arabic and Kordi breeds. The homozygous AA was not found, which was reported for the Sangsari (1%)[28], Chios (10%)[25] and Baluchi breeds with frequencies between 6 and 10%[25,27]. In all the aforementioned reports, the frequency of the wild genotype was higher than that of the heterozygous genotype (GA), which agrees with this report.

The values of Ho, He, F and EHW were only estimated for the FecG^{I} locus, because the FecX^{R} and FecG^{H} loci were monomorphic. A Ho value of 0.226±0.15 and a He value of 0.189±0.12 were found, which resulted in an F value of -0.194, indicating a non-significant excess of heterozygous individuals (p>0.05). Similar He values and excess heterozygotes occur in the breeds[19,23,27,28]. One of the reasons that would explain the excess of heterozygotes may be associated with the fact that said animals had greater prolificacy and, therefore, were allowed to continue reproducing in the sheep. No significant deviations from the EHW were found in the analyzed herd (p>0.05), which shows the lack of selection and breeding programs.

3.2 Factors that Affect Prolificacy

The descriptive statistics of the studied variables can be seen in Table 2. The average prolificacy was 1.3±0.3, with a maximum value of 2 offspring/female/parturition. This prolificacy value agrees with that reported by[5] (1.3±0.5 lambs/calving) for the same breed.

On average, each female had 2.4±1.6 births. The majority of females had a single birth (38.7%), 22.7%, 14%, 10%, 8% and 6.7% had two, three, four, five and six births, respectively. The females were serviced by six males, which serviced, on average, 25±16.3 females each. On average, in each season, 75±4.2 females were serviced for five years, with an average of 30±12.2 mounts/year.

Table 2. Descriptive statistics of the variables evaluated in the OPC breed

Variable	Mean	SD	Mínimum	Máximum	CV
Prolificity	1.3	0.3	1	2	23.1%
Birth of the mother	2.4	1.6	1	6	66.7%
Father	25	16.3	10	47	65.2%
Conception season	75	4.2	72	78	5.6%
Year of conception	30	12.2	21	48	40.1%

SD = standard deviation, CV = coefficient of variation

This is the first report of genetic-environmental factors that affect the natural prolificacy of OPC. An association analysis was only done for the FecG^{I} locus since it was the only polymorphic one; however, this polymorphism did not significantly affect the prolificacy value (p=0.087) (Table 3). Likewise, the other evaluated variables did not affect the natural prolificacy of the OPC (p>0.05) (Table 4).

Table 3. Average probity found for the genotype in the FecG^{I} locus

Genotype	N	Prolificity	Genotypic Frequency
GC	27	1.33 ± 0.36	0.22
GG	123	1.24 ± 0.30	0.78

Table 4. Effect of the number of calvings of the father and the season and year of conception on the natural prolificacy of the OPC

Number of lambings	Number of observations	Prolificity
1	58	1.26 ± 0.36
2	34	1.22 ± 0.25
3	21	1.23 ± 0,25
4	15	1.25 ± 0.26
5	12	1.27 ± 0,25
6	10	1.22 ± 0.25

Father	Number of observations	Prolificity
1	10	1.54 ± 0.30
2	47	1.12 ± 0.32
3	20	1.27 ± 0.24
4	15	1.23 ± 0.29
5	16	1.26 ± 0.28
6	12	1.29 ± 0.24
Conception season	**Number of observations**	**Prolificity**
Dry	72	1.24 ± 0.29
Rainy	78	1.25 ± 0.30
Year of conception	**Number of observations**	**Prolificity**
2012	21	1.31 ± 0.23
2013	19	1.27 ± 0.25
2014	37	1.24 ± 0.28
2015	48	1.20 ± 0.32
2016	25	1.27 ± 0.36

As in this report[16] found no effect of the genotype on the FecG^{I} locus on prolificacy; additionally[19] pointed out that animals with the AA genotype turn out to be sterile and, therefore, would be quickly eliminated from the animals. In this research, samples were only taken from individuals with a history of births, which could explain the absence of this genotype in the studied sample population.

In[25] reported an increase in prolificacy in the Chios breed, which has the AA genotype (2.25 lambs/calving), with respect to the other GA and GG genotypes (1.45 and 1.59, respectively)[14], for the Baluchi Indian breed, reported that the most prolific genotype is the heterozygous GA (1.38±0.05), with respect to the other two genotypes GG (1.23±0.03) and AA (1.03±0.05). Accordingly, there is no agreement on which genotype is the best. The variations in the prolificacy of these genotypes is probably due to the fact that the nucleotide substitution G260A is not synonymous and leads to a change of Arginine to Histidine at position 87 of the amino acid sequence and, although both amino acids are polar, negatively charged ions. The guanidinium group of Arginine results in a lower charge density[14].

In the present research, no significant association was found between the number of lamb parturitions and prolificacy although different authors have reported increases in the age of the animals[5,25,29]. A possible explanation for this phenomenon can be attributed to the age[30] and body weight of the sheep at the time of mating since, as the sheep mature and reach physical and physiological development, it becomes more efficient to maintain a pregnancy, produce more milk and express maternal ability[31]. The growth process competes with the gestation and reproductive processes by obtaining circulating nutrients in the organism, generating decreases in reproductive parameters. Thus, as in most tropical extensive production systems that have continuous mating, sheep are usually mated at between 20 and 26 kg; under these circumstances, the sheep take longer to recover their body condition after childbirth, affecting their reproductive rates, unlike intensive systems where the nutritional level is greater and nutrients optimize reproductive behavior[32].

The father did not affect the prolificacy of the female OPC, contrary to the findings of Cortes[33] for sheep on the Canary Islands and Sancristobal-Gaudy[34] for the Lacaune breed in France. These differences in the effect of the male can be closely related to the body condition, nutritional status, health status, male:female ratio in the mounts and the seasons, which have an impact on the sperm quality and reproductive function.

The two variables related to the date of conception of the offspring had no significant relationship with prolificacy although, in the rainy season of 2012, it was higher. In Pelibuey x Blackbelly sheep and their crosses with Dorper and Katahdin in Mexico, a significant effect of the year has been reported, but not for the season, on prolificacy[29]. In the Lacaune breed, an effect of the year[34] has also been reported for Canary Islands sheep kept in an intensive system, but not for the season (winter, spring, summer and autumn)[33].

4. Conclusions

The FecX^{R} and FecG^{H} polymorphisms were not found in the OPC. The allelic and genotypic frequencies and the He and F values found in the FecG^{I} locus were similar to those reported in other breeds around the world. The variation in the prolificacy was not explained by the variation of the FecG^{I} locus or by the non-genetic effects that were measured. It is necessary to extend this study to other candidate genes and/or polymorphisms.

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