Association of the FecB polymorphism with the natural prolificacy of the Colombian Creole Sheep

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ABSTRACT

Objective. Characterize and associate the FecB polymorphism with the natural prolificacy in the biotypes of Colombian Creole Sheep (OPC) Etíope and Sudán. Materials and methods. At 300 births from 167 OPC sheep, from the biotypes, Sudán (n = 73) and Etíope (n = 94), the effect of the FecB genotype was measured, and of the non-genetic factors: number of parturitions of the mother, the father, the season and the year of conception, on the natural prolificacy (litter size). For this, the animals were genotyped by PCR-RFLP (AvaII) for FecB and the productive records of the herd analyzed. The allelic and genotypic frequencies were calculated, which, together with the non-genetic factors, were associated with litter size using a fixed-effect GLM model. Results. The FecB\textsuperscript{B} allele presented lower frequency (0.379±0.152) than the FecB\textsuperscript{+} allele (0.622±0.152) for the whole OPC. These frequencies varied (p<0.05) between biotypes (Sudán: 0.486, Etíope: 0.271), the same occurred with the FecB\textsuperscript{BB} genotype (0.078 in Etíope and 0.236 in Sudán). The FecB\textsuperscript{++} genotype was more frequent in Etíope (0.526) and the heterozygous genotype in Sudán (0.5) and for the OPC (0.448±0.070). No significant differences were found between biotypes for non-genetic factors. The prolificacy varied (p<0.05) between biotypes (1.45±0.22 in Etíope and 1.34±0.03 in Sudán), with an average of 1.40±0.11 for the OPC. None of the non-genetic factors, as well as the FecB genotypes, affected the litter size of the OPC (p>0.05). However, this was higher in the FecB\textsuperscript{BB} genotype. Conclusions. The lucus studied was polymorphic. The litter size was not affected by non-genetic factors or the FecB genotype. These results can be used in assisted selection plans to increase OPC productivity.

Keywords: Allele frequency, BMPR1gene, genetic resources, (Source: CAB).
presentó menor frecuencia (0.379±0.152) que el alelo FecB+ (0.622±0.152) para todo el OPC. Estas frecuencias variaron (p<0.05) entre biotipos (Sudán: 0.486, Etiópe: 0.271), lo mismo ocurrió con el genotipo FecBB (0.078 en Etiópe y 0.236 en Sudán). El genotipo FecB++ fue más frecuente en Etiópe (0.526) y el genotipo heterocigoto en Sudán (0.5) y para el OPC (0.448±0.070). No se encontraron diferencias significativas entre biotipos para los factores no genéticos. La proliferidad varió (p<0.05) entre biotipos (1.45±0.22 en Etiópe y 1.34±0.03 en Sudán), con un promedio de 1.40±0.11 para el OPC. Ninguno de los factores no genéticos al igual que los genotipos FecB afectaron la proliferidad natural del OPC (p>0.05). Sin embargo, esta fue más alta en el genotipo FecBB++.

**Conclusiones.** El locus estudiado fue polimórfico. La proliferidad no se afectó por los factores no genéticos ni por el genotipo FecB. Estos resultados podrían ser utilizados en planes de selección asistida para aumentar la productividad del OPC.

**Palabras clave:** Frecuencias alélicas, gen BMPR1, recurso genético (Fuente: CAB).

**INTRODUCTION**

The main sheep breed in Colombia is called Criollo Creole Sheep (OPC), of which three biotypes can be established; Sudán, Etiópe and Abyssinian, with phenotypic differences based primarily on coat color and size (1,2). In Colombia, sheep production is carried out in traditional and family systems, with low input requirements, which are generally related to mixed production systems together with other species such as cattle and goats (3). The OPC is considered to be suitable for meat production in extensive systems, since the breed has a good adaptation to the productive conditions of the tropical climate, such as tolerance to heat, ectoparasites and the ability to consume low-value pastures nutritionally (4-6). Currently, the technological transfer to the sheep production system comes from places with different production conditions to the tropics (7). Thus, the genetic improvement in the OPC is based on crossings to take advantage of hybrid vigor (3,5,8) in characteristics associated with growth, but with little effect on the reproductive efficiency of the herd (9).

Reproductive efficiency can be measured through several parameters, including fertility, prolificacy and survival of the lamb. Prolificacy (number of lambs born over the total number of sheep) is the most studied parameter. Changes in prolificacy are mainly due to an increase in the ovulation rate, the number of fertilized eggs and embryonic survival, which causes an increase in the number of double and triple births (8,10).

The ovulation rate is determined by genetic and non-genetic effects. This last factor may include the nutrition of the lamb prior to puberty and service, the use of eating practices such as flushing, the female’s body condition, the period of time of the year of riding which it is directly linked to the availability of food, the chronological age of the female or the number of births and different hormonal treatments (10,11). Genetic factors include race, the effects of consanguinity and the action of single genes with a greater effect (12). These are called fertility genes (Fec) (13). Fec genes encode proteins of low molecular weight of regulatory function, both in the development of the ovary and the ovulation process (14). The three most studied Fec are the Growth Differentiation Factor 9 (GDF9) of which five polymorphisms called FecG, FecG, FecG, FecG and FecG, Bone Morphogenetic Protein 15 (BMP15) with eight described genetic variants (FecX, FecX, FecX, FecX, FecX, FecX, FecX and FecX) and Bone Morphogenetic Protein Receptor 1B (BMPR1B) of which only the phenotype called Boorola (FecB) has been described so far (8,15).

En el OPC se han estudiado los efectos de los polimorfismos genéticos FecR, FecG y FecG con la prolificidad. Sin embargo, se desconocen los posibles efectos del gen BMPR1B en la misma. Por tanto, el objetivo de este trabajo fue caracterizar y asociar el polimorfismo FecB con la prolificidad natural en los biotipos de OPC Etiópe y Sudán.

In the OPC the effects of the FecR, FecG and FecG genetic polymorphisms with prolificity have been studied (8,16). However, the possible effects of the BMPR1B-FecB gene on prolificity are unknown. Therefore, the objective of this work was to characterize and associate FecB polymorphism with natural prolificity in the Etiópe and Sudán OPC biotypes.
MATERIALS AND METHODS

Populations, blood collection, and DNA extraction. In the present investigation 167 Colombian Hair Sheep (OPC) was used, belonging to the biotypes, Sudán (n=73) and Etiøpe (n=94) of a herd located in the departments of Sucre, managed under conditions of continuous grazing in Bothriochloa pertusa and Braquiaria brizanta meadows, with availability of water and salt at will. Peripheral blood samples were obtained in tubes with anticoagulant (EDTA 7.2 mg) considering the procedures of sample collection, handling, and conservation, ethical, technical, scientific and administrative standards for research in animals contained in Law 84 (National Congress of Colombia, 1989). The DNA was extracted using the QIAamp® DNA Mini Kit commercial kit from QIAGEN. The quantity and quality of the DNA were evaluated using the NanoDrop 2000TM (Thermo Fisher Scientific).

Amplification and genotyping of the FecB locus. The PCR-RFLP technique was used to obtain genotypes. First, a 190pb fragment was amplified using primers F-5’-CCAGAGGACAATGCAAGAAGAAA-3’ and R-5’-CAAGATGTTTTCATGCTCATCAACAGGTC-3’ (17). The PCR reactions were carried out in a final volume of 25 µl containing 20 ng of DNA, 250 nM of each primer and 1X of the MangoMix™ super mix (Bioline®). The thermal profile included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30s, 60°C for 60s and 72°C for 60s. The amplifications were performed in an Eppendorf® MasterCycler Nexus Gradient thermal cycler. The visualization of the amplified fragments was performed in 1.2% agarose electrophoresis stained with GelRed™ (Biotium). In a final volume of 15 µl, containing 5 µl of the PCR product, 1U of the restriction endonuclease AvaII (G|GACC) and 1X of the buffer, the fragments of the previously amplified fragment were made. The reaction was incubated at 37°C for two hours, followed by 80°C for 20 minutes. Digestion products were visualized on 9% polyacrylamide gels (Acrylamide: Bis-acrylamide 37:1) run at 150 V for 40 minutes and stained with GelRed™ (Biotium). The homozygous FecB++ genotype only presented a fragment after incubation (190pb), FecBBB genotype two fragments (160pb and 30pb), while the heterozygous FecB+B genotype presented three fragments (190pb, 160pb and 30pb) (14,17,18).

Analysis of data. Genotypic and allelic frequencies were calculated for each biotype and in total (OPC). These frequencies were compared between biotypes using the Fisher test with a significance of 5%. All analyses were performed with the Arlequin programs ver 3.5.2.2 (19) and GENALEX ver 6.5 (20).

From the productive records of the herd, prolificity (litter size) was calculated in 300 births (Etiøpe n=145 and Sudán n=155), in addition to, number of parturitions for the mothers, the identification of the father, season and year of conception was counted as non-genetic effects. These data were analyzed using descriptive statistics and the difference between these values was evaluated using the R® minimum square method (21).

The prolificity observed in each biotype and for the OPC was associated with the FecB genotypes found using the fixed-effect GLM procedure, using R® (21) according to the following model:

\[ Y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + F_n + H_m + \varepsilon_{ijklmn} \]

Where:

- \( Y_{ijklmn} \) = prolificity observed
- \( \mu \) = Effect of the population mean
- \( A_i \) = Effect of the i-th genotype on the loci
- \( B_j \) = Effect of the j-th parturition of the mother
- \( C_k \) = Effect of the k-th father
- \( D_l \) = Effect of the l-th year of conception
- \( F_n \) = Effect of the n-th conception season
- \( H_m \) = Effect of the m-th Etiøpe or Sudán biotype
- \( \varepsilon_{ijklmn} \) = Effect of random error

RESULTS

The FecB variant in the OPC was polymorphic, with differences between biotypes (Table 1). The FecB+ allele showed a higher frequency in the Etiøpe biotype (p<0.05) and the FecB8 allele in the Sudán biotype (p<0.05). For the entire OPC population, the frequency of the FecB8 allele was lower (0.379±0.152). Significant differences (p<0.05) were found in the distribution of genotypes between OPC biotypes. The FecB++ genotype was the most frequent in Etiøpe, while the FecB++ genotype was the most common in Sudán and throughout the OPC. The genotype of interest FecB8 was more frequent in Sudán than in Etiøpe (p<0.05), with an average for the OPC of 0.157±0.111 (Table 1).
Allelic and genotypic frequencies for the FecB locus in the OPC.

<table>
<thead>
<tr>
<th>Biotype</th>
<th>Allelic Frequencies</th>
<th>Genotypic Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FecB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>FecB&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Etiope</td>
<td>0.729&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.271&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sudán</td>
<td>0.514&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.486&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OPC</td>
<td>0.622 ±0.152</td>
<td>0.379 ±0.152</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Different letters in the same column differ statistically (p<0.05).

In the variables number of parturition of the mother, father, season and year of conception, no statistical differences were found between the biotypes. Therefore, the descriptive statistics of these values are presented as averages for the OPC (Table 2). On average each female had 2.7±1.5 parturitions. The majority of females had only one birth (39.5%), 20.7%, 12%, 10%, and 9% had two, three, four and five parturitions respectively. The females were served by six males, which served on average 15±6.4 females each. On average, 70±5.6 females were served in five years at a time with an average of 35±11 mounts/year.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±S.D.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>CV&lt;sup&gt;o&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolificity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.40±0.11</td>
<td>1</td>
<td>2</td>
<td>7.9%</td>
</tr>
<tr>
<td>Mother’s parturition&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.7±1.5</td>
<td>1</td>
<td>5</td>
<td>55.6%</td>
</tr>
<tr>
<td>Father</td>
<td>15±6.4</td>
<td>10</td>
<td>25</td>
<td>42.7%</td>
</tr>
<tr>
<td>Season of conception&lt;sup&gt;d&lt;/sup&gt;</td>
<td>70±5.6</td>
<td>65</td>
<td>76</td>
<td>8.0%</td>
</tr>
<tr>
<td>Year of conception&lt;sup&gt;e&lt;/sup&gt;</td>
<td>35±11</td>
<td>27</td>
<td>48</td>
<td>31.4%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of lambs/female/parturition. <sup>b</sup> Mother’s parturition number. <sup>c</sup> Number of lambs per father. <sup>d</sup> Number of conceptions per season. <sup>e</sup> Number of conceptions per year. <sup>o</sup> Standard deviation. <sup>i</sup>Coefficient of variation.

The association between genotype and prolificacy was not significant (Table 3), although, this was greater in the FecB<sup>BB</sup> genotype in both biotypes and for the entire OPC, compared to the other genotypes.

Table 3. Average prolificity between FecB genotypes for each biotype.

<table>
<thead>
<tr>
<th>Biotype</th>
<th>Genotypes</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FecB&lt;sup&gt;++&lt;/sup&gt;</td>
<td>FecB&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Etiope</td>
<td>1.2±0.4</td>
<td>1.56±0.6</td>
</tr>
<tr>
<td>Sudán</td>
<td>1.33±0.5</td>
<td>1.32±0.4</td>
</tr>
<tr>
<td>OPC</td>
<td>1.27±0.09</td>
<td>1.44±0.1</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Different letters in the same column differ statistically (p<0.05).

DISCUSSION

The FecB variant consists in the exchange of an Adenine for a Guanine (c830A>G), which causes the non-synonymous change in position 249 of the protein, changing Glutamine to Arginine (Q249R) (14,15,22,23). This is the first report of the presence of the FecB<sup>a</sup> mutation in the OPC. In other breeds in the world, the frequency of this allele was 0.35 in Kalekoohi (22), 0.14 in Nilagiri (17), 0.61 in Garole (24), 1.0 in Hu (18), 0.63 in Han (18) and 0.5 in Bayanbulak (25). While this allele has not been found in the breeds Barbarine, Queue Fine de L’Ouest, Noire de Thibar, Sicilo-Sarde, D’man, Romanov, Finn, East Friesian, Teeswater, Blueface Leicester, D’Man, Chios, Mountain Sheep, German Whiteheaded Mutton, Lleyn, Loa, Galician, Barbados Blackbelly and St. Croix (18,16).

The size of the litter has a great impact on the reproductive efficiency of sheep. Prolificity is directly related to the ovulation rate (22) that is the result of the variation in the sensitivity of gonadotropin release and the feedback effects of gonadal steroids (27). These can be affected by both genetic (FecB) and non-genetic factors (8). The average prolificacy found in this research (1.40±0.11) was higher than that reported by other authors for this same racial group (4,8,28).

Several authors show a significant effect (p<0.01) in the increase of prolificacy in sheep with FecB<sup>BB</sup> genotype (15,18,22,23). Thus, the BMPR1B gene is one of the key candidate genes for the genetic control of the ovulation rate and the consequent increase in prolificacy in different sheep breeds. It has been shown that

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the mutated allele (FecB<sup>B</sup>) produces an increase in the ovulation rate, which additionally increases between +1 to +1.5 ovules released by each copy of the FecB<sup>B</sup> allele (29). Then, on average, sheep with FecB<sup>BB</sup> or heterozygous genotypes (FecB<sup>B+</sup>) have between 0.64 and 0.35 more lambs at birth, respectively, then sheep with FecB<sup>++</sup> genotype. The above makes the FecB<sup>B</sup> allele and the FecB<sup>BB</sup> genotype as objects of zootechnical interest.

In this work a significant association between genotype and prolificity was not found, however, in both biotypes and in general for the OPC the FecB<sup>BB</sup> genotype presented the greatest prolificity. Which represented 0.4 and 0.05 more lambs in Etiópe and Sudán, respectively. For the OPC, this increase in prolificacy was 14.7%.

The Sudán biotype showed a higher frequency of the allele and the genotype of interest (FecB<sup>B</sup> and FecB<sup>BB</sup>) compared to the Etiópe biotype (p<0.05), however, the prolificity observed was greater in the Etiópe biotype (p>0.05). The above suggests that the natural prolificacy for the OPC breed is surely under the control of other genes not determined so far. In this regard, the FecX<sup>R</sup>, FecG<sup>H</sup> and FecG<sup>I</sup> genetic polymorphisms (8,16) have been genotyped and associated with prolificity in the OPC, with no significant results.

As in this work, Pineda et al (8) report that the factors classified as non-genetic (parturitions number of the mother, father, of the season and year of conception) did not affect (p>0.05) the prolificacy in OPC population studied.

Several authors report that the natural prolificacy of the sheep increases with the age of the animal (4,30,31). A possible explanation for the phenomenon can be attributed to the age (32) and the bodyweight of the sheep at the time of mating, since as the sheep matures and reaches its body and physiological development, it becomes more efficient to maintain a gestation, produce more milk and express your maternal ability (33). Growth competes with pregnancy and reproductive processes for obtaining circulating nutrients in the body, which decreases reproductive parameters. That’s right, as is the case in most tropical extensive production systems, where the mating system is continuous and the sheep are usually paired between 20 and 26 kg (34).

The effect of the father did not affect the prolificity of the female OPC, these differences in the effect of the male, may be intimately related to the body condition, nutritional status, health status, the proportion of males:female in the herd and rest of the male between riding stations, which affect in one way or another on sperm quality and reproductive function (8.35).

The two variables related to the date of conception of the offspring had no significant relationship with prolificity, although the year 2014 and in the rainy season was greater. In Pelibuey x Blackbelly sheep and their crosses with Dorper and Katahdin in Mexico, they reported a significant effect of the year, but not of the time on prolificacy (31). In the Lacaune breed, it found effect of the year (35).

**Conflict of interests.**

The authors declare no conflict of interest with publication of this manuscript.

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