Madrid Gaviria, Stephania; López Herrera, Albeiro; Echeverri Zuluaga, José Julián
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Asociación entre un polimorfismo de FSHR con características productivas y reproductivas en el ganado Holstein de Antioquia

Stephania Madrid Gaviria[1*]; Albeiro López Herrera[1]; José Julián Echeverri Zuluaga[1]

ABSTRACT

Key words:
Bovine
FSH
PCR-RFLP
Productive traits
Reproductive traits
SNP

Because FSH and its receptor play a fundamental role in reproduction, the objective of this research was determining the effect of the A-320T polymorphism in productive and reproductive traits in Antioquia Holstein cows. The PCR-RFLP was used to amplify a segment of 970 bp of the bovine follicle stimulating hormone receptor gene (FSHR) which was digested with the restriction enzyme *Taq*I. The effect of the FSHR genotypes on productive and reproductive traits was determinate by a Mixed Linear Model and Tukey Test was used to establish significant differences between means for the three genotypes. The effect of allelic substitution was studied through a linear regression model where the genotypes AA, AT and TT were transformed into a quantitative scale of 0, 1 and 2, respectively according to the number of possessed T alleles. In Antioquia Holstein cattle the most common genotype was the AT (0.485) followed by TT (0.417) and AA (0.096) genotypes. Allele frequencies were 0.339 for A and 0.660 for T, respectively. The FSHR genotypes did not exert a significant effect on the principal productive parameters, except for fat percentage ($P<0.01$) where the TT individuals presented the highest percent. Results showed that T allele seems to improve the solids in milk while A allele improves dairy yield. The reproductive parameters were not affected by this SNP but AT animals showed a higher number of services per conception. Further studies are required to determine whether this SNP may be used as a molecular marker.

RESUMEN

Palabras claves:
Bovino
FSH
PCR-RFLP
Parámetros productivos
Parámetros reproductivos
SNP

Debido a que la FSH y su receptor cumplen un rol fundamental para la reproducción, el objetivo de este estudio fue determinar el efecto del polimorfismo A-320T en parámetros productivos y reproductivos en el ganado Holstein de Antioquia. Se utilizó la PCR-RFLP para amplificar un segmento de 970 pb del gen que codifica para el receptor de la hormona folículo estimulante (FSHR) que se digirió con la endonucleasa *Taq*I. El efecto de los genotipos de FSHR en los parámetros productivos y reproductivos se determinó mediante un Modelo Linear Mixto y se empleó la prueba de Tukey para establecer diferencias significativas entre las medias de los genotipos. El efecto de sustitución alélica se estudió mediante un modelo de regresión lineal donde los genotipos AA, AT y TT se transformaron a una escala de 0, 1 y 2 de acuerdo con el número de alelos T poseídos. Para el ganado Holstein de Antioquia el genotipo más común fue el AT (0,485) seguido por el TT (0,0417) y el AA (0,096). Las frecuencias alélicas fueron 0,339 y 0,660 para A y T. Los genotipos de FSHR no presentaron un efecto significativo en los parámetros productivos, excepto para el porcentaje de grasa (P<0,01) donde los individuos TT presentaron el mayor porcentaje. Los resultados mostraron que el alelo T parece mejorar el contenido de sólidos en leche, mientras que el alelo A mejora la producción de leche. Los parámetros reproductivos no se vieron afectados por este SNP pero los animales AT mostraron un mayor número de servicios por concepción. Se requieren más estudios para determinar si este SNP puede ser usado como marcador molecular.

[1] Grupo BIOGEM - Facultad de Ciencias Agrarias - Universidad Nacional de Colombia. A.A. 1779, Medellín, Colombia.
* Corresponding author <smadridg@unal.edu.co>

The profitability of dairy cattle systems is directly affected by productive and reproductive efficiency. The most important traits evaluated in these productive systems, such as, the performance dairy yield, compositional and sanitary status, weight gain, calving interval and others are affected not only by genetic and physiological factors, but also by environmental factors that exert a huge effect on the observed phenotype (Beuzen *et al.*, 2000).

Animal breeding programs are an alternative for improving the productive and reproductive parameters in subsequent generation; to achieve this goal, only the animals with higher genetic potential are used as progenitors. The marker assisted selection (MAS) is one of the most common strategies implemented to identify genetic differences between individuals. Molecular markers are sites in the genome where exists differences in the nucleotide sequence between individuals of the same species, resulting in a single nucleotide polymorphism (SNP) that can be identify through the SNP-RFLP (Restriction Fragment Length Polymorphism) technique (Deb *et al.*, 2012).

The follicle stimulating hormone (FSH), together with other hormones produced by the hypothalamic pituitary gonadal axis, has a fundamental role in oogenesis and spermatogenesis. In females FSH is responsible for recruiting ovarian follicles and allows the growth of the same, also induces aromatase activity on granulosa cells leading to the production of 17β-estradiol. Besides increases the production of inhibin to control its own synthesis (Assidi *et al.*, 2013; Medan *et al.*, 2007). The FSH is a glycoprotein formed by two different subunits; the α subunit is common to a family of closely related glycoproteins, but the β subunits is specific for each member of the family conferring these hormones a high degree of biological specificity, nevertheless both subunits are involved in the binding with the receptor (George *et al.*, 2011).

The FSH acts exclusively through its receptor, located in the surface of granulosa cells (Gromoll and Simoni, 2005). The FSH receptor (FSHR) belongs to the family of G protein coupled receptors, and its main function is to convert the extracellular stimuli on intracellular signals

(Kroeze *et al.*, 2003). This family of receptors shares a common structure with seven membrane spanning helices, an extracellular N-terminus and an intracellular C-terminus (Kroeze *et al.*, 2003). To transmit the signal, the intracellular domain interacts with heterotrimeric G protein activating the enzyme adenylyl cyclase to enhance the synthesis of the second messenger cAMP, which in turns activates protein kinasa A (Ulloa-Aguirre and Timossi, 1998).

The FSHR gene contains several SNP that may affect its activity. The number of SNPs varies with the species, in mice have been found 25 SNPs, while in humans have been reported more than 1.000 SNPs (Gromoll and Simoni, 2005; Ochsenkühn *et al.*, 2009). This polymorphism have been associated with ovarian function and delayed puberty in women (Layman *et al.*, 1997; Desai *et al.*, 2011). The SNP G-29A located in the promoter of the FSHR gene affects ovarian response in women undergoing *in vitro* fertilization treatments, with some genotypes presenting a lower response (Desai *et al.*, 2011). The effects of some polymorphism had also been studied in livestock. The SNP A-320T located upstream of the FSHR gene has been associated with sperm quality and response to superovulatory treatments in Chinese Holstein cattle (Yang *et al.*, 2010; Sang *et al.*, 2011), however there are no studies about the effect of this polymorphism on the main parameters of zootechnical interest. The objective of this study was to determine the effect of the A-320T polymorphism of FSHR gene in some productive and reproductive traits in Antioquia Holstein cows.

MATERIALS AND METHODS
Study population
Blood samples, productive and reproductive records to 1240 lactations from 356 dairy cows were used; those cows belonged to 9 dairy herds located in 6 different municipalities of Antioquia Department, which participates in a milk control program. Table 1 shows the distribution of the animals by herd and municipality.

DNA extraction and genotyping by PCR-RFLP
Genomic DNA was obtained from blood samples by the method of modified Salting out, described by Miller and coworkers (Miller *et al.*, 1988). To amplify a fragment of 970 bp on the 5' UTR of the FSHR which contains one

Table 1. Distribution of the animals studied by municipalities (N-Mun) and herd (N-Herd), and number of analyzed lactations by herd (Lac-N).

Municipality	N-Mun	Herd	N-Herd	Lac-N
Bello	27	H-1	27	121
Belmira	38	H-2	38	145
Entrerrios	61	H-3	45	184
		H-4	16	75
La Unión	57	H-5	57	105
Medellín	116	H-6	116	394
		H-7	33	118
San Pedro de los Milagros	57	H-8	14	62
		H-9	10	36

reported SNP of A-320T (GenBank number: rs43676359) were used the primers F: 5'-AGTTCGACCGCATCCCTG-3' y R: 5'-AATTCATTTGTGCCAGCATC-3' (Sang et al., 2011). The PCR was performed in a final reaction volume of 25 µL, containing 3.1 µL 10X reaction buffer (200 mM $(NH_4)_2SO_4$, 750 mM Tris-HCl pH 8.8 a 25°C), 2.7 mM of $MgCl_2$, 5 pmol primers, 0.24 mM of dNTP (deoxyribo nucleotide triphosphate), 1.5 unit of Taq-DNA polymerase and 100 ng of genomic DNA as template. The cycling protocol was at 95°C for 5 min, 35 cycles of 94°C for 30 s, 58°C annealing for 30 s, 72°C for 30 s, and 72°C for 8 min for the final extension. The digestion was carried out with 12 µL of PCR product, 7 U of *Taq*I and 1.6 µL of buffer in a final volume of 25 µL at 65°C for 16 hours. The amplified fragment contained four restriction sites (T´CG_A) for the enzyme, resulting in five fragments (446, 293, 140, 86 and 5 bp) for A allele. The T allele caused the loss of one restriction site, generating four fragments (586, 293, 86 and 5 bp). The digested products were visualized by 3% agarose gel electrophoresis.

Statistical analysis
Allelic and genotypic frequencies were determined with GenAlex Software (Peakall and Smouse, 2012).

Association of genotypes of FSHR with productive and reproductive performance
The analyzed parameters were milk yield adjusted to 305 days (AMY), protein (PP) and fat percentage (FT) as quality indicators and somatic cell count (SCC) as indicator of sanitary quality. The studied reproductive parameters were calving interval (CI) and service per conception (SPC). SCC was transformed to its logarithmic form according to the

formula (Rodriguez-Zas et al., 2000). In order to obtain more balanced data (SCS = 3+ log2(SCC/100) the births occurred since 1998 to 2003 were grouped in the year 2003, births occurred between 2004 and 2005 were grouped in 2005, and births from 2011 to 2014 were grouped in 2014. Besides the sixth to eleventh lactations were grouped on the sixth lactation.

The associations of genotypes of FSHR with productive and reproductive performance of Holstein cows were determined by a Mixed Linear Model. Tukey test was used to determine significance differences between the means obtained for the fixed effects included in the model. The statistical analyses were carried out with software SAS (Cary, 2009). The statistical model used was:

$$Y_{ijklmnopqr} = \mu + G_i + L_j + YB_k + MB_l + H_m + (H^*YB)_n + (H^*MB)_o + (YB^*MB)_p + (H^*YB^*MB)_q + A(G)_r + e_{ijklmnopqr}$$

Where $Y_{ijklmnopqr}$ is the dependent variable, which could be AMY, PP, FP, SCS, CI or SPC depending of the evaluated trait; μ is the mean for the characteristic in the population; G_i is the fixed effect of the FSHR genotype (1-3); L_j is the fixed effect of the lactation number (1-6); YB_k is the fixed effect of year of birth (1-8); MB_l is the fixed effect of month of birth (1-12); H_m is the fixed effect of the herd (1-9); $(H^*YB)_n$ is the fixed effect of the interaction between herd and year of birth; $(H^*MB)_o$ is the fixed effect of the interaction between herd and month of birth; $(YB^*MB)_p$ is the fixed effect of the interaction between year and month of birth; $(H^*YB^*MB)_p$ is the fixed effect of the interaction between herd, year and month of birth; $A(G)_r$ is the random effect of genotype nested on the animal and $e_{ijklmnopqr}$ is the random residual error. Besides these

effects, the real milk yield (RMY) and days in milk (DIM) were included as covariates in the model for PP y FT. For SCS, CI and SPC also was included the covariate AMY, additionally for SPC was included the CI, corresponding to the analyzed lactation, as a covariate.

Allelic substitution analysis

A regression analysis was performed to find out the effect of allelic substitution. The genotypes were converted into 0, 1 or 2 according to the number of possessed T alleles. The regression model used was the following:

$$Y_j = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + e_j$$

Where Y_i is the dependent variable value in function of the number of G alleles; β_0 is the intercept; β_1 is the linear regression coefficient estimated for allele substitution; X_i number of T alleles possessed by the animal i (0, 1 or 2); β_2 is the linear regression coefficient between milk yield corrected by the effect of herd and lactation number (CMY) and the dependent variable; X_2 is the value for the independent variable CMY (not included for the AMY model) and e_i is the random residual error. For all analyzes a significance level of 0.05 was used.

RESULTS AND DISCUSSION
Descriptive analysis

The mean for AMY for Antioquia Holstein population was 5588 ± 1492 L/lactation, with 3.06 ± 0.26 and 3.89 ± 0.46 percentages of protein and fat respectively. The mean for CI was 414 ± 85 with 1.67 ± 1.17 SPC. The descriptive analysis for each trait is shown in Table 2.

The average found for milk production is higher than the value reported by (Rodríguez *et al.*, 2013) who reported a

Table 2. Mean, standard deviation (S.D), variation coefficient (V.C) and number of observations (N) for adjusted milk yield (AMY), protein percentage (PP), fat percentage (FP), somatic cell score (SCS), calving interval (CI) and service per conception (SPC) for Antioquia Holstein cattle.

| Evaluated trait		Mean	S.D	V.C	N
AMY	(L/lac)	5588	1492	26.70	811
PP	(%)	3.06	0.26	8.56	421
FP	(%)	3.89	0.46	12.04	421
SCS		4.19	1.01	24.13	413
CI	(days)	414	85	20.53	876
SPC		1.67	1.17	69.85	1091

mean for milk production of 5039 Kg. On the other hand the values for PP, FP and SCS are lower than the values reported for those authors who found 3.12% PP, 3.97% FP and a mean of 4.72 for SCS for the Antioquia Holstein cattle (Rodríguez *et al.*, 2013). In 2011, Echeverri and collaborators reported for that population lower values for milk yield and fat percentage, 4482 L/lac and 3.37% respectively, but in that research it was found a higher value for protein percentage, 3.16% The mean for SPC is consistent with the average reported in a recent study of our investigation group, which reported a mean of 1.66 SPC, but the value reported for CI, 435 days, was a little higher than the value reported on this research (Madrid and Echeverri, 2014).

Allelic and genotypic frequencies

The PCR product of 970 bp corresponding to a fragment of the 5' UTR of the FSHR gene contained four restriction sites for the enzyme *Taq*I when the allele A was present, and three restriction sites if the T allele was present, resulting in five or four fragments, respectively, after digestion with the enzyme. The PCR product and the restriction patterns are shown in Figure 1.

For the population of Antioquia Holstein cattle the T allele was the most common (0.660) while the A allele had a minor frequency (0.339). The genotypes TT and AT showed a similar frequency, 0.417 and 0.485 respectively. The AA genotype had the lower frequency with 0.096. The
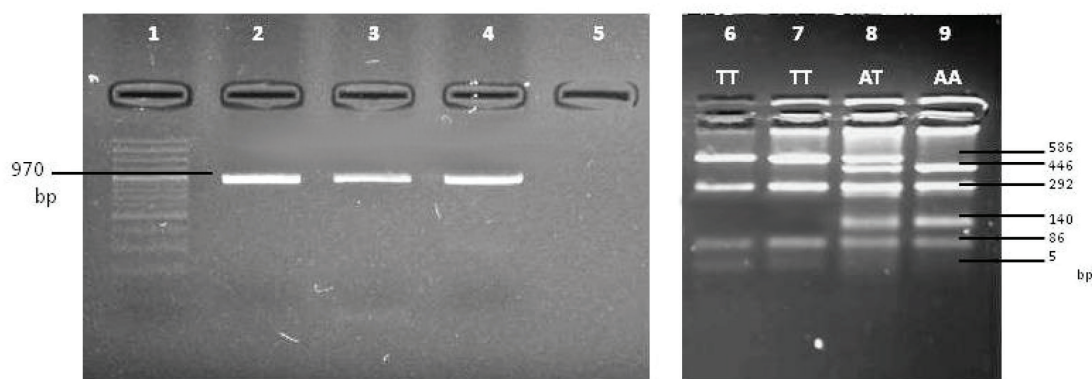
Figure 1. Amplified fragment (970 bp) of the follicle stimulating hormone receptor gene (lanes 2 to 4) and restriction pattern after digestion with TaqI enzyme for each genotype (TT lanes 6 and 8, AT lane 8 and AA lane 9); lane 1 correspond to the molecular weight marker, and lane 5 correspond to the negative control (PCR without DNA template).

allele frequencies found for Antioquia Holstein cattle are very similar to the frequencies found for Chinese Holstein bulls, 0.321 for A allele and 0.679 for T allele, despite this, the genotype frequencies are not as similar. For Chinese Holstein bulls the most common genotype was the TT with a frequency of 0.496, followed by the AT genotype with frequency of 0.366, but in both population the AA genotype presented the lower frequency, 0.138 for Chinese population (Sang *et al.*, 2011). This polymorphism has also been analyzed in Chinese Holstein cows, the allele frequency reported was 0.301 and 0.699 for A and T allele respectively, and the genotypes frequencies found were 0.119, 0.364 and 0.517 for AA, AT and TT genotypes respectively, for this population the TT genotype was also the most common (Yang *et al.*, 2010)

Association of FSHR genotypes with dairy traits
Productive and sanitary traits
Milk yield. The L, H, MB, the interactions H*YB, H*MB and H*YB*MB had a highly significant effect ($P<0.01$), the interaction YB*MB also had a significant effect ($P<0.05$) on AMY. The FSHR genotype and other variables did not show a significant effect ($P>0.05$) on this trait. Means by genotype are shown in Table 3. The regression coefficient between CMY and the FSHR genotype was -201.05 ($P<0.01$) (Table 4), meaning that the change of an A allele for a T allele reduced milk production on 201 L/lactation ($P<0.01$). *Protein percentage.* The variables RMY and DIM presented a significant effect ($P<0.01$) on PP. Any other variable, including genotype, presented a significant effect ($P>0.05$) on PP. Average for PP by genotypes are

Table 3. Tukey's averages for evaluated traits‡ according to follicle stimulating hormone receptor (FSHR) genotype for Antioquia Holstein cattle.

Evaluated Trait	FSHR Genotype		
	AA	AT	TT
AMY (L)	5902*	5709	5521*
PP (%)	2.98†	3.01*	3.07*†
FP (%)	3.78†	3.89*	4.00*†
SCS	4.24	4.16	4.37
CI (days)	403	427	412
SPC	1.67*	2.26*	2.04

*† Significant difference according Tukey test
‡ Adjusted milk yield (AMY), protein and fat percentage (PP, FP), somatic cell score (SCS), calving interval (CI) and service per conception (SPC)

shown in Table 3. The regression coefficient between PP and FSHR genotypes was 0.01 (*P*>0.05) (Table 4) showing that PP increases on 0.01% when there is a change of one A allele for a T allele.

Fat percentage. RMY, H, YB and the interaction H*YB showed a significant effect (*P*<0.05) on FT. The genotype for FSHR also showed a highly significant effect (*P*<0.01). According to Tukey test the most favorable

Table 4. Intercept (I), regression coefficient between FSHR genotype and the dependent variable (β)‡ and their significance (P value) for the allelic substitution analysis for Holstein dairy cattle of Antioquia.

Dependent Variable	I	B	P value
CMY(L/lac)	5687.14	-201.05	0.0070
PP (%)	3.36	0.019	0.3266
FP (%)	4.16	0.070	0.0763
SCS	4.70	0.103	0.2163
CI (days)	357.30	3.244	0.6255
SPC	-0.167	0.130	0.1853

‡ Corrected milk yield (CMY), protein percentage (PP), fat percentage (FP), somatic cell score (SCS), calving interval (CI) and service per conception (SPC)

genotype was TT with 0.11% higher fat content than AT animals, and 0.22% higher fat content than AA animals. The averages for this trait by the genotypes are shown in Table 3. The regression coefficient between FP and FSHR genotypes was 0.07 (*P*>0.05) (Table 4), this means that FP increased on 0.07% with the substitution of an A allele for a T allele.

Although there is no biological reason for the association between the SNP A-320T and milk yield or the percentage of solids in it, since this receptor is only expressed in the surface of granulosa cells (Simoni *et al.*, 1997; Ulloa-Aguirre and Timossi, 1998; Gromoll and Simoni, 2005), the found results could be interpreted from the lactation duration and reproductive efficiency. Although this polymorphism showed no significant effect on calving interval and services per conception, it can be seen that individuals with AA genotype had the lowest values for these traits. Achieve a new pregnancy in less time, decreases open days and lactation duration, especially of the last third which is characterized by showing the lowest level of production, this way, shorter lactations present a higher value to be set to 305 days in milk. On the other hand, cows with TT genotype had a higher number of services per conception and calving interval, because of this, the last third of lactation is prolonged maintaining a relative low level of production compared to the level obtained at the peak of production that occurs in the first third of lactation, so a lower value

of milk production is obtained when set the real milk yield to a duration of 305 days.

The effect of the SNP A-302T on protein and fat percentage is explained from the volume of milk yield and its negative correlation with percentage of solids in it (Calvache and Navas, 2012). For AA cows that showed a higher milk yield it was evidenced a greater dilution effect of the solids in milk that is why these individual showed the lowest percentage of fat and protein, while TT individuals that presented a lower milk yield also presented a higher percentage of protein and fat due to a smaller dilution effect.

Somatic cell score

For this traits the L and H presented a significant effect (*P*<0.01). The others variables included in the model had no significant effect (*P*>0.05). Means according genotype are shown in Table 3. The regression coefficient between SCS and FSHR genotype was 0.10 (*P*>0.05) (Table 4) meaning that the change of an A allele for a T allele causes an augmentation of 0.10 on the SCS. For this trait the heterozygous animals presented the lowest score, since the SCS reflects the sanitary quality of milk a lower score indicates better quality, this way it seems that heterozygous animals are apparently less susceptible to infections that lead to increased SCC and the SCS as consequence (Rodriguez-Zas *et al.*, 2000).

Reproductive traits
Calving interval

None of the variables included in the model, including the genotype, showed a significant effect on this trait ($P>0.05$). Means according to genotype are shown in Table 3. Regression coefficient between CI and FSHR genotypes was 3.2 ($P>0.05$) (Table 4), indicating that the substitution of an A allele for a T allele causes and increment of 3 days between births.

Service per conception

The CI and the interaction YB*MB presented a highly significant effect ($P<0.01$), while YB presented a significant effect ($P<0.05$) on this trait. Genotype or any other variable showed a significant effect ($P>0.05$). Means according genotype are shown in Table 3. The regression coefficient between CI and FSHR genotype was 0.13 ($P>0.05$) (Table 4), this means that the number of SPC increases on 0.13 with the change of an A allele to a T allele.

Contrary to superiority of the heterozygous regarding SCS, for the reproductive parameters, it cannot be determined that one of these alleles of the FSHR gene enhances the reproductive parameters. Nevertheless, these results showed that heterozygous animals had a tendency to a lower reproductive efficiency, since they presented the largest interval between births and the highest number of services to get pregnant.

This SNP has also been associated with semen quality Chinese Holstein bulls. In 2011, Sang and coworkers evaluated the effect of this polymorphism of the FSHR gene in traits like semen volume per ejaculate, sperm concentration, motility, sperm motility in frozen semen, acrosome integrity rate and abnormal sperm rate. These investigators found that bulls with AA genotype presented a significantly higher volume per ejaculate and higher sperm concentration in comparison with bulls with AT genotype. Nevertheless this polymorphism had no any other significant correlation with the evaluated traits (Sang et al., 2011).

Because of the important role of the FSH receptor in reproduction this gene has been proposed as a candidate gene that may affect the superovulatory response in cows. To corroborate this hypothesis, the effect of the A-320T SNP on the number of unfertilized ova, number of degenerate embryos, number of transferable embryos and total number of ova was evaluated in a population of Chinese Holstein cows. On this research it was found that the evaluated polymorphism did not presented a significant association with any superovulation traits, and neither showed a significant dominant or additive effect (Yang et al., 2010).

The effect of other polymorphisms on this receptor has been studied. In the coding regions of the bovine FSHR gene have been detected three non-synonymous mutations (C337G, A871G and C1973G). The GG Holstein cows at position 337 presented a higher percentage of viable embryos. The SNPs A871G and C1973G also affect superovulatory response, for these SNPs the AA and GG individuals presented lower embryo yield (Cory et al., 2013) For *Bos taurus* x *Bos indicus* beef cattle it has been evaluated the association of a SNP in the exon 10 of the FSHR gene with sexual precocity measured as the probability of pregnancy at first breeding. In this research it was found that heterozygous heifers showed a higher pregnancy rate, but the genotype had no significant effect on the evaluated characteristic (Marson et al., 2008).

In women the polymorphism on the FSHR gene can also affect the ovarian response and can lead to premature ovarian failure (Cordts et al., 2011). A reported SNP (G-29A) in this gene is associated with alterations in the level of expression of the receptor in granulose cells. The 72% of patients with AA genotype presented a poor ovarian response to the *in vitro* fertilization treatment, possibly caused by a difference in the DNA-protein binding affinity. Also the AA individuals presented a relative lower expression of FSHR at mRNA and protein level compared with the GG individuals (Desai et al., 2011).

For the Holstein cattle of Antioquia it had also been studied the effect of other polymorphisms in productive and reproductive traits. Some polymorphisms in the major histocompatibility complex or Bovine Leukocyte Antigen (BoLA) have been associated with susceptibility or resistance to infectious diseases and are related to susceptibility to mastitis; in Antioquia Holstein cattle the alleles DRB3.2*8 and DRB3.2*14 are associated with subclinical mastitis, while the allele DRB3.2*33 is associated with resistance to subclinical mastitis (Zambrano et al., 2011). BGH gene is associated with reproductive parameters in Antioquia Holstein cattle. This

gene affects age at first service, age at first calving, age at first postpartum service and age at second calving, for all of these traits the genotype (-/-) is the most inefficient, presenting the highest values for those characteristics (Arango, 2012). Other SNPs affect the productive parameters. The change of a cytosine for a thymine on the intron 6 of the lactoferrin gene may affect the PP and SCS. Regarding PP the best genotype was BB, while for SCS the AB genotype was the most favorable since reported the lowest score (Rodríguez *et al.*, 2013). Others genes like kappa casein, prolactin and bovine growth hormone (BGH) have shown to have a significant effect on PP, AMY and FP, respectively with some genotypes improving milk production and the quality of the same (Echeverri *et al.*, 2013).

CONCLUSIONS

Since the A-320T SNP did not show a significant effect on the main productive and reproductive parameters, this gene cannot be considered as one of the major genes that affect the characteristics of economic interest for dairy cattle systems.

It is noteworthy that productive traits, and especially the reproductive traits are multifactorial characteristics, this means that they are affected by many factors, not only genetic and physiological but also environmental, and each of these factors is responsible for a small percentage of the observed variation in each characteristic. This is why is important to continue with the studies to identify the effect of the FSHR A-320T polymorphism in productive and reproductive parameters, not only for Holstein cows but also in other breeds or species, to determine the utility of this gene as a molecular marker and include it in breeding programs.

REFERENCES

Arango, J. 2012. Asociación del polimorfismo de intrón 3 de la hormona de crecimiento bovino (BGH) con parámetros de crecimiento y reproductivos en novillas Holstein del Departamento de Antioquia. Tesis Magister en Biotecnología. Facultad de Ciencias. Universidad Nacional de Colombia, Medellín. 92 p.

Assidi, M., F.J., Richard and M.A. Sirard. 2013. FSH in vitro versus LH in vivo: similar genomic effects on the cumulus. Journal of Ovarian Research 6(68): 1-13. doi: 10.1186/1757-2215-6-68

Beuzen, N., M. Stear and K. Chang. 2000. Molecular markers and their use in animal breeding. The Veterinary Journal 160(1): 42–52. doi: org/10.1053/tvjl.2000.0468

Calvache, I. and A. Navas. 2012. Factores que influyen en la composición nutricional de la leche. Revista Ciencia Animal 5: 73-85.

Cary, N. 2009. SAS/STAT 9.2 Useris Guide. SAS Institute Inc.

Cordts, E., D. Christofolini, A. dos Santos, B. Bianco and C Barbosa. 2011. Genetic aspects of premature ovarian failure: a literature review. Archives of Gynecology and Obstetrics 283(3): 635–43. doi: 10.1007/s00404-010-1815-4

Cory, A., C. Price, R, Lefebvre and M. Palin. 2013. Identification of single nucleotide polymorphisms in the bovine follicle-stimulating hormone receptor and effects of genotypes on superovulatory response traits. Animal Genetics 44(2): 197–201. doi: 10.1111/j.1365-2052.2012.02380.x

Deb, R., S. Chakraborty and U. Singh. 2012. Molecular Markers and Their Application in Livestock Genomic Research. Journal of Veterinary Science & Technology 03(04): 7579. doi: 10.4172/2157-7579.1000e106

Desai, S., S. Achrekar, B. Pathak, S. Desai, V. Mangoli, R. Mangoli and S. Mahale. 2011. Follicle-stimulating hormone receptor polymorphism (G-29A) is associated with altered level of receptor expression in Granulosa cells. The Journal of Clinical Endocrinology and Metabolism 96(9): 2805–2812. doi: 10.1210/jc.2011-1064

Echeverri, J., J. Arango and J. Parra. 2011. Análisis comparativo de los grupos genéticos Holstein, Jersey y algunos de sus cruces en un hato lechero del norte de Antioquia en Colombia. Actas Iberoamericanas de Conservación Animal 1: 264–267.

Echeverri, J., A. López and L. López. 2013. Asociación de tres SNPs con algunas características productivas y de calidad de la leche en hatos Holstein en Antioquia, Colombia. Revista EIA 10(20): 63–72.

George, J.W., E. Dille and L. Heckert. 2011. Current concepts of Follicle-Stimulating Hormone Receptor gene regulation. Biology of Reproduction 84: 7-17. doi: 10.1095/biolreprod.110.085043

Gromoll, J. and M. Simoni. 2005. Genetic complexity of FSH receptor function. Trends in Endocrinology and Metabolism 16(8): 368–373. doi: 10.1016/j.tem.2005.05.011

Kroeze, W., D. Sheffler and B. Roth. 2003. G-protein-coupled receptors at a glance. Journal of Cell Science 116(24): 4867–4869. doi: 10.1242/jcs.00902

Layman, L., E. Lee, D. Peak, A. Namnoum, K. Vu, B. Van Lingen, M. Gray, P. McDonough, R. Reindollar and J. Jameson. 1998. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone B-subunit gene. The New England Journal of Medicine 337(9): 607–611. doi: 10.1097/00006254-199802000-00016

Madrid, S. and J. Echeverri. 2014. Association between conformation traits and reproductive traits in Holstein cows in the department of Antioquia - Colombia. Revista Facultad Nacional Agronomia Medellín 67(2): 7311–7319. doi: 10.15446/rfnam.v67n2.44174

Marson, E., J. Ferraz, F. Meirelles, J. Balieiro and J. Eler. 2008. Effects of polymorphisms of LHR and FSHR genes on sexual precocity in a *Bos taurus* x *Bos indicus* beef composite population. Genetics and Molecular Research 7(1): 243–251. doi: 10.4238/vol7-1gmr418

Medan, M., K. Arai, G. Watanabe and K. Taya. 2007. Inhibin: Regulation of reproductive function and practical use in females. Animal Science Journal 78(1): 16–27. doi: 10.1111/j.1740-0929.2006.00399.x

Miller, S., D. Dykes and H. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16(3): 1215. doi: 10.1093/nar/16.3.1215

Ochsenkühn, R., V. Von Shönfeldt and M. Simoni. 2009. FSH receptor polymorphisms and ovarian function. Médecine de la Reproduction, Gynécologie Endocrinologie 11(4): 265–270.

Peakall, R. and P. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics Oxford, England) 28(19): 2537–2539. doi: 10.1093/bioinformatics/bts460

Rodríguez, N., A. López and J. Echeverri. 2013. Asociación del polimorfismo del intrón 6 en el gen lactoferrina bovino con características de importancia en producción lechera. Revista Lasallista de Investigación 10(2): 9–16.

Rodriguez-Zas, S., D. Gianola and G. Shook. 2000. Evaluation of models for somatic cell score lactation patterns in Holsteins. Livestock Production Science 67(1-2): 19–30. doi: 10.1016/s0301-6226(00)00193-7

Sang, L., Q. Du, W. Yang, K. Tang, J. Yu, G. Hau, X. Zhang and L. Yang. 2011. Polymorphisms in follicle stimulation hormone receptor, inhibin alpha, inhibin bata A, and prolactin genes, and their association with sperm quality in Chinese Holstein bulls. Animal reproduction science 126(3-4): 151–156. doi: 10.1016/j.anireprosci.2011.04.023

Simoni, M., J. Gromoll and E. Nieschlag. 1997. The follicle-stimulating hormone receptor: Biochemistry, molecular biology, physiology, and pathophysiology. Endocrine Reviews 16(6): 739-773. doi: 10.1210/er.18.6.739

Ulloa-Aguirre, A. and C. Timossi. 1998. Structure-function relationship of follicle-stimulating hormone and its receptor. Human reproduction update 4(3): 260–283. doi: 0.1093/humupd/4.3.260

Yang, W., S. Li, K. Tang, G. Hua, C. Zhang, J. Yu, L. Han and L. Yang. 2010. Polymorphisms in the 5' upstream region of the FSH receptor gene, and their association with superovulation traits in Chinese Holstein cows. Animal reproduction science 119: 172–177. doi: 10.1016/j.anireprosci.2010.02.004

Zambrano, J., J. Echeverri and A. López. 2011. Alleles of the BoLA DRB3.2 gene are associated with mastitis in dairy cows. Revista Colombiana de Ciencias Pecuarias 24: 145–156.