

INHA A192G polymorphism and its association with dairy traits in Antioquia Holstein cattle

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ADDITIONAL KEYWORDS

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Inhibin.
PCR-RFLP.
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SNP.

SUMMARY

The objective of this research was determining the effect of the A192G polymorphism in some productive and reproductive traits in Antioquia Holstein cows. To achieve this, the technique PCR-RFLP was used to amplify a segment of 249 bp of the bovine inhibin alpha gene (INHA) which was digested with the restriction enzyme *MspI*. The effect of the INHA genotypes on the productive and reproductive traits was determined 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, AG and GG were transformed into a quantitative scale of 0, 1 and 2, respectively according to the number of possessed G alleles. In Antioquia Holstein cattle the most common genotype was the AG (0.529) followed by AA (0.269) and GG (0.201) genotypes. Allele frequencies were 0.534 for A and 0.466 for G. The INHA genotypes did not exert a significant effect on the principal productive parameters, except for fat percentage where GG animals presented the highest value for this trait. Despite it was not a significant effect of this polymorphism on reproductive parameters, Tukey test showed that G allele tend to have a detrimental effect on female fertility, increasing the calving interval and the number of services per conception, decreasing the reproductive efficiency of the herds. Further studies are required to determine whether this SNP may be used as a molecular marker.

Polimorfismo A192G de INHA y su asociación con características lecheras en ganado Holstein de Antioquia

RESUMEN

El objetivo de esta investigación fue determinar el efecto del polimorfismo A192G en algunos parámetros productivos y reproductivos en vacas Holstein de Antioquia. Para esto, se utilizó la técnica PCR-RFLP para amplificar un segmento de 249 pb del gen de inhibina alfa bovina (INHA) el cual fue digerido con la enzima de restricción *MspI*. El efecto de los genotipos de INHA en las características productivas y reproductivas se determinó con un Modelo Lineal Mixto y la prueba de Tukey se usó para establecer diferencias significativas entre las medias de los tres genotipos. El efecto de sustitución alélica se estudió a través de un modelo de regresión lineal donde los genotipos AA, AG y GG fueron transformados a una escala cuantitativa de 0, 1 y 2, respectivamente de acuerdo al número de alelos G poseídos. En el ganado Holstein de Antioquia el genotipo más común fue el AG (0,529) seguido por el AA (0,269) y el genotipo GG (0,201). Las frecuencias alélicas fueron 0,534 para A y 0,466 para G. Los genotipos de INHA no ejercieron un efecto significativo en los principales parámetros reproductivos, excepto para porcentaje de grasa donde los animales GG presentaron el mayor valor para esta característica. A pesar de que no hubo un efecto significativo de este polimorfismo en los parámetros reproductivos, la prueba de Tukey mostró que el alelo G tiende a tener un efecto negativo en la fertilidad de las hembras, aumentando el intervalo entre partos y el número de servicios por concepción, disminuyendo la eficiencia reproductiva de los hatos. Se requieren más estudios para determinar si estos SNP pueden ser utilizados como marcadores moleculares.

PALABRAS CLAVE ADICIONALES

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INTRODUCTION

Productive and reproductive performances are some of the most important parameters in dairy cattle since they directly affect profitability of these productive systems. Many of the characteristics evaluated in animal production systems like milk yield, weight gain, litter size, calving interval and others are influenced by several factors; environmental, physiological and genetics (Beuzen *et al.*, 2000).

In order to improve the efficiency of dairy herds, farmers are interested in knowing which animals have greater genetic potential to implement animal breeding programs that allow the improvement of the parameters of economic interest in the next generations.

One of the most common strategies used in gene selection is the marker-assisted selection (MAS). 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 identified through the PCR-RFLP (Restriction Fragment Length Polymorphism) technique (Deb *et al.*, 2012).

The hypothalamic-pituitary-gonadal axis is the main physiological route affecting the bovine estrous cycle. The gonadotropin-releasing hormone (GnRH) produced by the hypothalamus, regulates the synthesis and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary; these hormones stimulate gametogenesis and steroidogenesis. Finally, the hormones produced by the gonads like inhibin and estradiol, regulate the hypothalamic-pituitary function controlling the reproductive activity (Parraguez *et al.*, 2012). Inhibin is a peptide hormone produced by granulosa cells in the ovary that regulates the levels of FSH and inhibits its production affecting folliculogenesis (Bhardwaj *et al.*, 2012). The mature protein is formed by two different subunits, the alpha and beta subunit, which are linked by two disulfide bonds. Because the β subunit has two possible forms; β A and β B, and both of them can bind to the α subunit, exists two forms for this hormone, inhibin A and inhibin B (Magoffin and Jakimiuk, 1997), which are secreted at different times of the estrous cycle. Inhibin B secretion is higher at the beginning of the follicular phase, while the production of inhibin A increases in the second half of the cycle, indicating its production by the preovulatory follicle (Harris *et al.*, 2005; Medan *et al.*, 2007). The mechanism whereby the inhibin regulates the action of FSH can be considered endocrine and paracrine, since not only has a negative feedback to suppress FSH secretion from the pituitary, but also regulates the FSH receptor expression in the ovary by suppressing the promoter activity of this gene (Lu *et al.*, 2009).

Since both subunits of this hormone have been related with reproductive performance in animals and reproductive diseases in humans there have been several studies on the effect of some polymorphisms in these traits. The inhibin alpha gene (INHA) has been proposed as a candidate for premature ovarian failure (POF) in humans; patients with this syndrome lose ovarian function before the age 40 (Shelling *et al.*, 2000; Harris *et al.*, 2005). In cattle has been reported a change of an adenine for a guanine at position 192 (A192G) on the exon 2 of the INHA, this SNP is considered a synonymous mutation, this means that the change of an adenine for a guanine does not change the amino acid sequence of the protein, since both codons (CGA and CGG) encode the amino acid Arginine (NCBI, 2013), but result in an additional restriction site for the enzyme *MspI*. In Chinese Holstein cattle has been found that this SNP is associated with response to superovulatory treatments (Tang *et al.*, 2011) and semen quality (Sang *et al.*, 2011), and the G allele exerts a positive effect in both characteristics. However there are no studies about the effect of this polymorphism on the main parameters of zootechnical interest. The objective of this research was to determine the effect of the A192G polymorphism of INHA gene in some productive and reproductive parameters in Antioquia Holstein cows.

Table I. Distribution of the studied animals by municipalities and herd (N), and number of analyzed lactations by herd (Lac N) (Distribución de los animales estudiados por municipio y hato (N), y número de lactancias analizadas por hato (Lac N)).

Municipality	N	Herd	N	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

MATERIAL AND METHODS

STUDY POPULATION

Blood samples, productive and reproductive records corresponding 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 I** 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 249pb on the INHA exon 1 which contains one reported SNP of A192G (GenBank number: rs41257116) were used the primers F: 5'-GCCCTGTTTCTGGATGCC-3' and R: 5'-ATTCAACCCAACCTGCCTA-3' (Tang *et al.*, 2011). The PCR was performed in a final reaction volume of 30 μ l, containing 3.8 μ l 10X reaction buffer (200 mM $(\text{NH}_4)_2\text{SO}_4$, 750 mM Tris-HCl pH 8.8 at 25°C), 2.5 mM of MgCl_2 , 15 pmol of both primers, 0.4 mM of dNTP (deoxyribo nucleotide triphosphate), 1.5 unit of Taq-DNA polymerase (Thermo Scientific) and 100 ng of genomic DNA as template. The cycling protocol was 5 min at 95°C, 34 cycles of 94°C for 45 s, 62°C annealing for 45 s, 72°C for 45 s, and 72°C for 10 min for the final extension. The digestion was carried out with 15 μ l of PCR product, 7 U of *MspI* and 2.0 μ l of buffer in a final volume of 30 μ l for 8 hours at 37°C. The amplified fragment contained two common and one mutant restriction sites (GGGAC) for the enzyme at positions 31, 154 and 75, respectively, resulting in three fragments (123, 95 and 31 bp) for A allele and four fragments (95, 79, 44 and 31 bp) for G allele. The digested products were visualized by 4% agarose gel electrophoresis.

STATISTICAL ANALYSIS

ALLELIC AND GENOTYPIC FREQUENCIES

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

ASSOCIATION OF GENOTYPES OF INHA 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 $SCS = 3 + \log_2 (SCC/100)$ (Rodríguez-Zas *et al.*, 2000). In order to obtain more balanced data, 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 INHA 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 (SAS Institute Inc. Cary, NC 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:

$Y_{ijklmnopqr}$ = the dependent variable, which could be AMY, PP, FP, SCS, CI or SPC depending of the evaluated trait;

μ = the mean for the characteristic in the population;

G_i = the fixed effect of the INHA genotype (1-3);

L_j = the fixed effect of the lactation number (1-6);

YB_k = the fixed effect of year of birth (1-8);

MB_l = the fixed effect of month of birth (1-12);

H_m = the fixed effect of the herd (1-9);

$(H*YB)_n$ = the fixed effect of the interaction between herd and year of birth;

$(H*MB)_o$ = the fixed effect of the interaction between herd and month of birth;

$(YB*MB)_p$ = the fixed effect of the interaction between year and month of birth;

$(H*YB*MB)_q$ = the fixed effect of the interaction between herd, year and month of birth;

$A(G)_r$ = the random effect of genotype nested on the animal and

$e_{ijklmnopqr}$ = 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 G alleles. The regression model used was the following:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + e_i$$

Where:

Y_i = the dependent variable value in function of the number of G alleles;

β_0 = the intercept;

β_1 = the linear regression coefficient estimated for allele substitution;

X_1 = number of G alleles possessed by the animal i (0, 1 or 2);

β_2 = the linear regression coefficient between milk yield corrected by the effect of herd and lactation number (CMY) and the dependent variable;

X_2 = the value for the independent variable CMY (not included for the AMY model) and e_i is the random residual error.

RESULTS

DESCRIPTIVE ANALYSIS

The mean for AMY for Antioquia's Holstein population was 5.588 ± 1.492 L/lactation, with 3.06 ± 0.26 and 3.89 ± 0.46 percentage 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 II**.

ALLELIC AND GENOTYPIC FREQUENCIES

The PCR product of 249 bp corresponding to a fragment of the exon 1 of INHA gene presented two common and one mutant restriction site for the enzyme *MspI* resulting in three or four fragments for A and G alleles, respectively as shown in **figure 1**.

Table II. Mean, standard deviation (SD), variation coefficient (VC) 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 (Media, desviación estándar (SD), coeficiente de variación (VC) y número de observaciones (N) para producción de leche ajustada (AMY), porcentaje de proteína (PP), porcentaje de grasa (FP), puntaje de células somáticas (SCS), intervalo entre partos (CI) y servicios por concepción (SPC) para el ganado Holstein de Antioquia).

Evaluated trait	Mean	SD	VC	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	4.54	1.34	29.69	413
CI (days)	414	85	20.53	876
SPC	1.67	1.17	69.85	1091

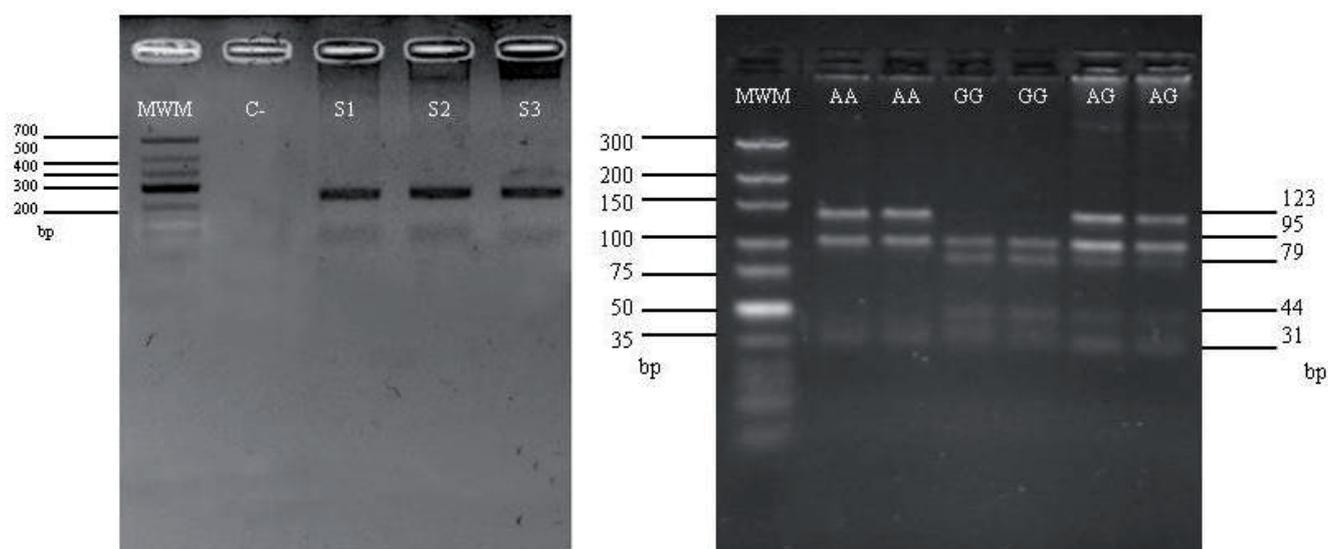


Figure 1. Amplified fragment (249 bp) of the inhibin alpha gene (lanes 3 to 5) and restriction pattern after digestion with MspI enzyme for each genotype (AA lanes 7 and 8, GG lanes 9 and 10, AG lanes 11 and 12); lanes 1 and 6 correspond to the molecular weight marker and lane 2 correspond to the negative control (PCR without DNA template) (Fragmento amplificado (249pb) del gen de inhibina alfa (líneas 3 a 5) y patrón de restricción luego de la digestión con la enzima MspI para cada genotipo (AA líneas 7 y 8, GG líneas 9 y 10, Ag líneas 11 y 12); las líneas 1 y 6 corresponden al marcador de peso molecular y la línea 2 corresponde al control negativo (PCR sin ADN muestra)).

The most common genotype on Antioquia's Holstein population was the heterozygous AG (52.92%), both homozygous genotypes presented similar frequencies, 26.95 and 20.13% for AA and GG, respectively. The allele frequency was 53.4% for A allele and 46.6% for G allele.

ASSOCIATION OF INHA GENOTYPES WITH DAIRY TRAITS

PRODUCTIVE AND SANITARY TRAITS

MILK YIELD

The L, H, YB, the interactions H*YB, H*MB and H*YB*MB had a highly significant effect ($p < 0.01$) on AMY. The INHA genotype and other variables had no significant effect on this parameter ($p > 0.05$). Means for each genotype are shown in **table III**.

PROTEIN PERCENTAGE

Protein percentage was significantly affected by RMY ($p < 0.01$), the others effects, including the genotype, were no significant ($p > 0.05$). Means for each genotype are shown in **table III**.

FAT PERCENTAGE

RMY, YB and INHA showed a significant effect on FP ($p < 0.05$). Other variables included in the model showed no significant effect ($p > 0.05$). According to Tukey test the most favorable genotype was GG with a value of 0.01% more fat than other genotypes. Means for each genotype are shown in **table III**.

SOMATIC CELL SCORE

SCS was significantly affected by L and H ($p < 0.01$). Other effects, including the genotype, had no significant effect in this parameter of sanitary quality of milk ($p > 0.05$). Means for each genotype are shown in **table III**.

REPRODUCTIVE TRAITS

CALVING INTERVAL

The interaction YB*MB had a significant effect on CI ($p < 0.05$). Any other variable included in the model, even INHA genotype, had a significant effect on the characteristic ($p > 0.05$). Means for each genotype are shown in **table III**.

SERVICES PER CONCEPTION

The number of SPC was significantly affected by H, YB*MB and CI ($p > 0.01$) and by H*MB ($p < 0.05$). The INHA genotype and the others variables included in the model had no significant effect ($p > 0.05$). Means for each genotype are shown in **table III**.

Table III. Tukey averages by inhibin alpha (INHA) genotype 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 (Medias de Tukey para los genotipos de inhibina alfa (INHA) para producción de leche ajustada (AMY), porcentaje de proteína (PP), porcentaje de grasa (FP), puntaje de células somáticas (SCS), intervalo entre partos (CI) y servicios por concepción (SPC) para el ganado Holstein de Antioquia).

Evaluated trait	INHA g genotype		
	AA	AG	GG
AMY (L)	5512	5631	5485
PP (%)	3.07	3.04	3.01
FP (%)	3.89	3.89***	3.90***
SCS	4.24	4.13	4.26
CI (days)	402***	413	434***
SPC	1.68	2.00	1.78

***Significative difference according Tukey test.

ALLELIC SUBSTITUTION ANALYSIS

For the milk yield corrected by the herd and lactation number the change of one A allele to a G allele increases milk yield on 98 L/lactation ($p>0.05$). The PP increases on 0.01% ($p>0.05$) while FP decreases almost the same magnitude in response to allelic substitution. SCS increases when there is a change of A to G allele in a magnitude of 0.012 ($p>0.05$). Respective to the reproductive parameters, CI increases 15 days ($p<0.01$) and the number of SPC augment 0.03 ($p>0.05$) units when an A allele mutates to a G allele. The intercept, regression coefficient and their significances for each regression model are shown in **table IV**.

DISCUSSION

The mean for milk yield was higher than the value reported in other investigations for Antioquia's Holstein population, in which the average for this trait was 4.482 L/lactation (Echeverri *et al.*, 2011). However Corrales and coworkers found a higher value (7.155 L/lactation) for milk yield on the dairy cattle of Antioquia (Corrales *et al.*, 2011). The PP value reported in this study was lower than the one found by other researchers (3.16%), on the other hand, the mean for FP was higher than the value reported on literature (3.37%) (Echeverri *et al.*, 2011). Respective to the reproductive parameters, the average for SPC was almost the same, while the CI was lower than the average reported by our research group in a recent investigation; the reported values were 1.67 for SPC and 453 days for CI (Madrid and Echeverri, 2014).

For a Chinese population of Holstein cows the allele frequency was 55 and 45% for the A and G allele respectively. The genotype frequencies for this population were 21.18% for AA genotype, 67.79% for AG and 11.01% for GG genotypes (Tang *et al.*, 2011). Although the allele frequency was very similar between Chinese and Antioquia Holstein population, in the Chinese population the AG genotype was more common among the animals, while the GG genotype had a minor frequency than in Antioquia Holstein population.

Within the population of Chinese Holstein bulls there has also been studied the presence of the A192G SNP. Unlike the Chinese and Antioquia Holstein cows, the most common genotype for the Chinese Holstein bulls was AA, followed by the heterozygous and by last the homozygous GG, with a frequency of 53.7, 30.9 and 15.4% respectively (Sang *et al.*, 2011).

The different forms of the INHA gene had no significant effect on the main milk traits evaluated in dairy herds. SPC and CI were not affected by the INHA genotypes. Nevertheless Tukey test showed that some genotypes of INHA tend to improve the fertility of cows decreasing time between calving, this way, cows with AA genotype can get pregnant after delivery more easily than cows with GG genotype. Although the Tukey test showed no significant differences, GG individuals have a higher number of SPC than AA individuals, thus increasing open days and hence CI. The allelic substitution analysis also shows that for every change of an A allele to a G allele the CI increases 15 days

Table IV. Intercept (I), regression coefficient between INHA genotype and the dependent variable (β) and their significance (p value) for the allelic substitution analysis for CMY, protein percentage (PP), fat percentage (FP), somatic cell score (SCS), calving interval (CI) and service per conception (SPC) for Antioquia Holstein dairy cattle (Intercepto (I), coeficiente de regresión entre el genotipo de INHA y la variable dependiente (β) y su significancia (p value) para el análisis de sustitución alélica para producción de leche corregida (CMY), porcentaje de proteína (PP), porcentaje de grasa (FP), puntaje de células somáticas (SCS), intervalo entre partos (CI) y servicios por concepción (SPC) para el ganado lechero Holstein de Antioquia).

Dependent variable	I	β	p value
CMY(L/lac)	5315.62	98.76	0.1754
PP (%)	3.35	-0.015	0.4446
FP (%)	4.30	0.012	0.7399
SCS	4.65	0.012	0.881
CI (days)	337.66	15.80	0.0065
SPC	0.39	0.032	0.7110

($p<0.01$). According to these results the G allele reduces female fertility reducing reproductive efficiency of the herds.

The effect of this polymorphism has also been studied in Chinese Holstein bulls and cows, but related with sperm quality and response to superovulatory treatments. In Chinese Holstein bulls it was studied the association of the INHA A192G SNP with sperm quality traits like volume per ejaculate, sperm concentration, motility, sperm motility in frozen semen, acrosome integrity rate and abnormal sperm rate. This polymorphism only has a significant effect on acrosome integrity rate; bulls with AA genotype had lower value for this trait than the GG genotype (Sang *et al.*, 2011). In Holstein females was evaluated the association of this SNP with superovulatory response. These investigators found that cows with GG genotype presented a significant increase in the number of ova than AG and AA genotypes during the first three superovulation treatments, the GG animals also produced more transferable embryos than animals with other genotypes. Cows without superovulation response had genotypes AA or AG. In both studies the G allele presented a favorable effect on the evaluated traits (Tang *et al.*, 2011). This result differs from the findings in this research where the G allele shows apparent negative effect on female fertility.

For the Chinese Holstein population it was suggested that although the SNP A192G does not change the amino acid sequence of the protein it may affect the expression of the INHA gene and the stability of the transcript, resulting in a lower negative feedback effect that increase the circulating concentration of FSH, improving the reproductive performance (Tang *et al.*, 2011; Sang *et al.*, 2011).

The effect of some polymorphisms on the inhibin genes has also been study to find possible applications in livestock. In Boer does the SNP A651G located on the exon 2 of the INHA gene has shown to have a significant effect on litter size of second parity does; thereby the heterozygous animals have 0.27 times more

offspring than those individuals with homozygous genotype (Wu *et al.*, 2009). Another polymorphism that affects litter size in goats is the A782G of the inhibin beta B (INHBB) gene. In Jining Grey goats the females with AA genotype have larger litters than those with genotype AB or BB (Chu *et al.*, 2012).

The genes encoding both subunits of the hormone inhibin has been proposed as candidate genes associated with certain reproductive disorders in women. The polymorphism INHA C16T has been studied to identify its relation with POF. It was found that T allele is underrepresented in the population with POF; this allele is related with a shorter TG repeat in the INHA promoter but had no significant effect on the promoter activity, however another haplotype of this promoter had no activity at all. This way, the INHA promoter haplotypes can lead to develop POF (Harris *et al.*, 2005). Another polymorphism on this gene has been associated with POF, the mutation G769A generates the change of an alanine for a threonine in the aminoacid sequence of the protein that may affect the protein ability to join with his receptor, resulting in an inability to activate the signal transduction pathway (Shelling *et al.*, 2000).

For Antioquia Holstein cattle, it had also been studied the effect of other polymorphisms in productive and reproductive traits. The change of a cytosine for a thymine on the intron 6 of the lactoferrina 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). The BGH is also 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 polymorphisms have been associated with susceptibility to mastitis; the mayor histocompatibility complex or Bovine Leukocyte Antigen (BoLA) is related with susceptibility or resistance to infectious diseases, 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).

CONCLUSION

Together with the above genes, gene INHA is part of the group of major genes that can affect the characteristics of economic importance in animal production systems. It is very important to note that most of the productive and reproductive traits evaluated in animal production systems are multifactorial, this means that are affected by a large number of variables, genetic and environmental, that can modulate the observed phenotype. This is why is important to continue with the studies to identify the effect of the INHA A192G poly-

morphism 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.

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