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Allelic Variants of FSHR Gene in Cows of Different Genotypes in Mexico

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Abstract: The allelic variants of FSH-Receptor (FSHR) gene in cows of different genotypes was determined. Forty-five cows were allotted in groups according to genotype: *Bos taurus* (n = 15), *Bos indicus* (n = 15) and *Bos taurus* x *Bos indicus* (crossbred; n = 15). The hardy-weinberg equilibrium was determined in the three populations. The gene shown alleles A (243, 63 bp), B (306 bp) and C (243, 150 bp). In the *Bos indicus* herd, frequencies for genotypes AA and AB were 69 and 28%, respectively, whereas frequencies for alleles A and B were 83 and 17%. In the crossbred herd, frequencies for genotypes AA and AB were 75 and 23%, respectively and frequencies for alleles A and B were 87 and 13%. In the *Bos taurus* herd, frequencies for genotypes AA, AB and CC were 3, 1 and 64%, respectively, whereas frequencies for alleles A, B and C were 17, 3 and 80%. Hardy-Weinberg equilibrium was found in the *Bos indicus* and crossbred herds ($p>0.05$), but not in the *Bos taurus* herd and in the population as a whole ($p<0.05$).

Key words: Allelic variant, *Bos taurus*, *Bos indicus*, cattle, FSHR gene, molecular polymorphism

INTRODUCTION

The Follicle-Stimulating Hormone (FSH) starts and maintains follicular development by binding to its specific Receptor (FSHR) in the surface of the granulosa cells in the ovary (Dierich *et al*., 1998; Simoni *et al*., 1997). This binding allows the activation of the FSHR codifying gene (Simoni *et al*., 1997). The FSHR gene in *Bos taurus* cattle was studied by Houde *et al*. (1994), this gene is located in chromosome 11 and its structure is determined by 10 exons and 11 introns; the first 9 exons enclose the extracellular domain, whereas exon 10 encloses the transmembrane domain (Houde *et al*., 1994; Simoni *et al*., 1997).

The existence of allelic variants in FSHR gene reported in humans (Kerkelä *et al*., 2007; Simoni *et al*., 1997, 1999; Wunsch *et al*., 2007) and cattle (Almeida *et al*., 2000; Marson *et al*., 2005, 2008; Rahal *et al*., 2000; Tambasco *et al*., 2000), indicates that the FSHR gene is polymorphic. These changes in the molecular structure of the FSHR gene cause desensitization of the FSH receptors in the cell membrane, which results in a less efficient hormone signal transmission (Gromoll *et al*., 1996; Huhtaniemi and Aittomaki, 1998). In cattle, the importance of the identification of DNA polymorphisms lies in their relation with productive and reproductive genotypes (Allan *et al*., 2007; Tambasco *et al*., 2000; Vasconcellos *et al*., 2003). To this respect, Rahal *et al*. (2000) found two polymorphic regions in Nelore cows that lead to mutations related to productive genotypes. Latronico and Arnhold (2006) reported that the changes in the DNA sequence can affect the activation of FSHR gene and they also, indicated that the genotype plays an important role in ovarian physiology.

In the Mexican tropics, the bovine herds are commonly made up of *Bos taurus*, *Bos indicus* and *Bos taurus* x *Bos indicus* crossbred animals. Thus, the characterization of the allelic variability of the FSHR gene in different cattle breeds allows to take advantage of heterozygosis and select individuals that are carriers of specific loci of reproductive importance (Marson *et al*., 2005).

Therefore, the objective of the present study was to determine the allelic variants of the FSHR gene in *Bos taurus*, *Bos indicus* and *Bos taurus* x *Bos indicus* cows in the Mexican humid tropic.

MATERIALS AND METHODS

Location and experimental animals: The study was carried out in two bovine commercial farms, one of purebred *Bos indicus* and purebred *Bos taurus* cattle

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Table 1: Oligonucleotide primers used for FSHR[1] gene6

RFLP[2]	Chromosome	Exon	Sequence (5'-3')[3]	Reference
AluI	11	10	F: CTGCCTCCCTCAAGGTGCCCCTC R: AGTTCTTGGCTAAATGTCTTAGGGGG	Houde *et al.* (1994)

[1]Follicle-stimulating hormone receptor. [2]Restriction fragment length polymorphism; [3]F (forward)-5'-3' direction, R (reverse)- 3'-5' direction

and other of *Bos taurus* × *Bos indicus* crossbred cattle, both located in Veracruz, Mexico, under tropical climate conditions. The cows in the two farms were kept under similar management and sanitary conditions, in rotational grazing systems. Forty-five cows were included in the study and were allotted into three groups according to their genotype as follows: *Bos taurus* (European Type Brown Swiss; n = 15), *Bos indicus* (Nelore and Indo-Brazilian; n = 15) and *Bos taurus* x *Bos indicus* (crossbred; ¾ Holstein ¼ Zebu and ¾ American Brown Swiss ¼ Zebu; n = 15).

Isolation of genomic DNA: From each of the cows, blood samples were collected into sterile vacutainer tubes added with disodium EDTA. The genomic DNA was isolated from leukocytes through proteinase K treatment, followed by the addition of isopropanol for DNA precipitation (Sambrook and Russell, 2001). The genotypes for the FSHR gene polymorphism were determined by PCR-RFLP and the restriction endonuclease AluI (Marson *et al*., 2005).

DNA amplification: DNA was amplified by PCR using the pair of oligonucleotide primers indicated by Marson *et al*. (2005) (Table 1). Each PCR reaction was made in a final reaction volume of 25 μL, containing 10× PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl; Biogenicas®, Cd. de Mexico, Mexico), 2.0 mM $MgCl_2$ (Promega®, Madison, WI, USA), 0.5 mM of each dNTP (deoxynucleotide triphosphate, Promega®), 0.4 μM of forward primer, 0.4 μM of reverse primer and 1 unit of Taq DNA polymerase (Promega®), according to the protocol by Marson *et al*. (2005). The amplification reactions were made in a MiniOpticon Real-Time PCR Detection System (Bio-Rad®, Hercules, CA, USA) and consisted of one cycle of 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 58°C for 30 sec and 72°C for 2 min, with a final extension at 72°C for 10 min. Verification of DNA amplification was made by electrophoresis in a 1.8% agarose gel (Promega®), stained with 1 μL ethidium bromide (0.5 μg mL^{-1}) in 1% Trisborate-EDTA (TBE) buffer. The products of the amplifications were digested with the specific restriction enzyme AluI (New England BioLabs® Inc., Ipswich, MA, USA), using 10 μL of each of the amplified products in a final volume of 20 μL; 5 U of AluI and 9.5 μL 10× NE Buffer 2 (New England BioLabs®) were added to each sample. The digestion was verified by electrophoresis in a 2.5% agarose gel (Promega®). Visualization of the fragments of the restriction sites was made using a UV transilluminator UltraLum® (UltraLum Incorporated, Claremont, CA, USA) and the image analyzer software GeneSnap (Syngene®, Synoptics Ltd., Cambridge, England).

Statistical analysis: The analyzed gene was considered to have three alleles: A-C. The genotypic and allelic frequencies for the FSHR gene were determined by direct counting, using a frequency table. The genetic variability of the population was evaluated through the observed number of alleles and the observed and expected heterozygosity for the gene. To determine, if the population was in equilibrium for the locus (Hardy-Weinberg equilibrium), the observed and expected genotypic frequencies, for each of the genotypes and for the whole population, were compared through the χ^2 test, significant values ($p<0.05$) rejected H_0 (H_0, Hardy-Weinberg equilibrium) and non-significant values ($p>0.05$) accepted H_0 (Smith-Keary, 1975). The properties of the populations in study were analyzed using the following formula:

Presence of two alleles (Suarez *et al*., 2001):

$$(p+q)^2 = p^2 + 2pq + q^2$$

Presence of three alleles (Nicholas, 1998):

$$(p + q + r)^2 = p^2 + q^2 + r^2 + 2pq + 2pr + 2qr$$

RESULTS AND DISCUSSION

Genotyping: Electrophoretic visualization of the band resulting from DNA amplification shown the expected fragment of 306 bp in *Bos taurus* and *Bos indicus* sp.

The digestion of the amplification products allowed to obtain alleles A (2 bands; 243 bp, 63 bp), B (3 bands; 306 bp) and C (2 bands; 243 bp and 150 bp) and revealed the presence of genotypes AA, AB and CC, being AA and AB for the *Bos indicus* and *Bos taurus* × *Bos indicus* cows, respectively and CC for the *Bos taurus* cows.

Genotypic and allelic frequencies: Alleles A and B were present in the three genotypes, whereas allele C was present only in the *Bos taurus* individuals. The observed genotypic frequencies for the FSHR gene for the population as a whole were 0.51(51%), 0.22(22%) and 0.27(27%) for genotypes AA, AB and CC, respectively.

The allelic frequencies for the whole population were 0.62% for allele A, 0.11(11%) for allele B and 0.27(27%) for allele C. In the *Bos indicus* herd, the frequencies of genotypes AA, AB and CC were 0.69(69%), 0.28(28%) and 0(0%), respectively. The frequencies for alleles A-C were 0.83(83%), 0.17(17%) and 0(0%), respectively.

In the *Bos taurus* x *Bos indicus* group, the frequencies of genotypes AA, AB and CC were 0.75(75%), 0.23(23%) and 0.0(0%), respectively. The frequencies for alleles A-C were 0.87(87%), 0.13(13%) and 0.0(0%), respectively.

In the *Bos taurus* cows the presence of alleles A-C was observed. The genotype frequencies were 0.03% for AA, 0.01(1%) for AB and 0.64(64%) for CC. Allele frequencies were 0.17(17%) for allele A, 0.03(13%) for allele B and 0.80(80%) for allele C.

Hardy-Weinberg equilibrium: The Hardy-Weinberg equilibrium was not observed for the population as a whole ($p<0.05$). The observed and expected heterozygosis for the whole population are shown in Table 2. The Hardy-Weinberg equilibrium was observed in the *Bos indicus* and *Bos taurus* x *Bos indicus* herds ($p>0.05$), but not in the *Bos taurus* group ($p<0.05$). The observed and expected heterozygosis for the *Bos indicus*, *Bos taurus* x *Bos indicus* and *Bos taurus* genotypes are shown in Table 3.

The results shown the presence of three alleles for the FSHR gene, contrary to previous reports of only two alleles in cattle (Campagnari, 2002; Loss *et al*., 2008; Marson *et al*., 2005). According to Buxade (1995), each gene can have two or more alleles, known as allelic series that arise as a result of mutations thus, the same gene in different individuals can experience different mutations that originate a new allele (Audesirk and Audesirk, 2008). Allele A (243 and 63 bp) found in the *Bos indicus* influenced cattle in the present study is the same that was reported by Marson *et al*. (2005) as allele C (243 and 63 bp) in Zebu-hybrid cattle in Brazil. This finding suggests that this molecular weight could be characteristic of the Zebu breeds developed in South and Central America, which originated from the crossbreeding of different Zebu breeds brought in from India and Spain (Bradley *et al*., 1996; Sanders, 1980).

The 3 genotypes of the present study shown a higher frequency of homozygosis for alleles A (*Bos indicus* and *Bos taurus* x *Bos indicus* herds) and C (*Bos taurus* herd). The genetic basis for heterozygosis lies in the fact that different breeds originate from different alleles for a single character, many of them probably in homozygous condition, due to processes of adaptation to local conditions, as well as to random factors of changes in the allelic frequencies (genetic drift) (Charlesworth, 2009). The

Table 2: Observed heterozygosis (H_S) and expected heterozygosis (H_E) for the whole population

Genotype	AA	BB	CC	AB	AC	BC	Total
H_S	23	0	10	12	0	0	45
H_E	17	1	3	6	15	3	45
Obtained χ^{2*}	1.80	0.6	24.2	2.3	14.9	2.6	46.4**
χ^2 value from distribution table (3 df, $\alpha = 0.05$)							7.81

*$\alpha = 0.05$ **$p<0.05$

higher frequency of allele A, which is characteristic of Zebu cattle in the *Bos taurus* x *Bos indicus* crossbred herd in this study could indicate a predominance of *Bos indicus* genotype in these animals. Likewise, even though the *Bos taurus* herd shown a high proportion of homozygosis for allele C, allele A was also present. The presence of fragments that are characteristic of Zebu cattle in the *Bos taurus* group of this study can be explained because, this herd was originally of *Bos indicus* genotype, but crossbreeding and genetic selection were practiced to increase the proportion of *Bos taurus* genes and as a result of a process of introgression between *Bos taurus* and *Bos indicus* individuals, it ultimately became a purebred *Bos taurus* herd. Therefore, genetic selection probably did not affect the expected allelic variability in the *Bos indicus* and *Bos taurus* × *Bos indicus* herds, but there was an important intervention of systematic factors in the formation of the *Bos taurus* group.

The Hardy-Weinberg equilibrium law states that in a large random intra breeding population, not subjected to excessive selection or mutation, the gene and genotype frequencies will remain constant over time, provided immigration, mutation and selection do not take place. The Hardy-Weinberg equilibrium found in the *Bos indicus* herd might indicate that in this population the breeding process has been at random in the absence of systematic factors that could affect its genetic behavior and as a result, it has remained stable regarding the allelic and genotypic frequencies.

Thus, this herd has maintained its genetic diversity by natural selection pressure (Sanchez-Aparicio *et al*., 2007). On the contrary, the absence of Hardy-Weinberg equilibrium in the *Bos taurus* herd might have been due to the high proportion of homozygosis for genotype CC and might suggest some degree of introgression, as was mentioned above. This is in accordance to the statements of Tambasco *et al*. (2000) and Vasconcellos *et al*. (2003) that events such as accumulation of some genotypes, subdivision of the population, mutations, selection, migration, or endogamy can result in a state of lack of equilibrium in the population. Moreover, Gardner *et al*. (2002) and Wunsch *et al*. (2007) indicated that when,

Table 3: Observed Heterozygosis (HS) and Expected Heterozygosis (HE) for each of the three genotypes

Genotype	AA	BB	CC	AB	AC	BC	Total
Bos indicus							
H_S	10	0	0	5	0	0	15
H_E	10	0	0	4	0	0	15
Obtained χ^{2*}	0.167	0	0.416	0.166	0	0	0.60**
χ^{*} value from distribution table (1 df, $\alpha = 0.05$)	-	-	-	-	-	-	3.84
Bos taurus × ***Bos indicus***							
H_S	11	0	0	4	0	0	15
H_E	11.3	0	0.3	3.5	0	0	15
Obtained χ^{2*}	0.006	0	0.266	0.082	0	0	0.4**
χ^{2} value from distribution table (1 df, $\alpha = 0.05$)	-	-	-	-	-	-	3.84
Bos taurus							
H_S	2	0	12	1	0	0	15
H_E	0.4	0.0	9.6	0.2	4	0.8	15.6
Obtained χ^{2*}	6.0167	0.0167	0.6000	4.1667	4.000	0.800	15.6***
χ^{2} value from distribution table (3 df, $\alpha = 0.05$)	-	-	-	-	-	-	7.81

*$\alpha = 0.05$; **$p>0.05$; ***$p<0.05$

systematic factors and a strong selection pressure participate in the formation of any herd, the Hardy-Weinberg equilibrium is affected.

The finding of three allelic variants for the FSHR gene in this study is in accordance with some reports that shown the existence of polymorphisms in this gene in humans (Kerkelä *et al*., 2007; Simoni *et al*., 1999; Wunsch *et al*., 2007) and cattle (Almeida *et al*., 2000; Marson *et al*., 2005, 2008; Rahal *et al*., 2000; Tambasco *et al*., 2000).

The characteristics of the FSHR gene have an important role in ovarian stimulation and the knowledge of its physiology can be used to predict differences in the function of the FSHR and the ovarian response to FSH (Simoni *et al*., 1997). Moreover, a common polymorphism in the FSHR gene was associated with human ovarian response to gonadotropins in some studies (Behre *et al*., 2005; Sudo *et al*., 2002) but not in others (d'Alva *et al*., 2005). This has not been studied in cattle, but it would be important to determine the ovarian response to FSH of each allele found in the FSHR gene in this study.

CONCLUSION

In the present study, the characterization of allelic variants of the FSHR gene for different cattle genotypes contributed to the knowledge of the behavior of this gene under tropical climate conditions. Further studies need to be carried out to determine the ovarian response to FSH for each of these allelic variants in cattle, since this information would be very valuable for multiple ovulation and embryo transfer programs, as it would allow to select the females that are carriers of the desired allele for maximum response to FSH in order to improve the reproductive performance of bovine herds.

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