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Genetic Characterization of Indigenous Sudanese Cattle Using *FSHR* and *LHR* Genes

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Abstract

The aim of this study was to estimate the genotypic and allelic frequencies of the two polymorphisms located in the receptor of Follicle Stimulating Hormone (FSHR) and Luteinizing Hormone (LHR). One hundred and sixteen blood samples were collected from three native Sudanese dairy ecotypes. The studied samples include 32, 34 and 50 cows from Butana, Kenana and Erashy cattle types, respectively. The DNA was extracted following standard methods. The purified DNA was subjected to PCR-RFLP techniques to identify polymorphisms of the *FSHR* and *LHR* genes in the three Sudanese cattle ecotypes. The amplified fragments of *FSHR* (306 bp) were digested with restriction enzymes *ALu* resulting in 243 and 63 bp fragments. At exon10in the *FSHR* gene, all genotyped cows in the investigated native Sudanese population were homozygous for AA genotype. The restriction endonuclease *Hhal* allowed the identification of three genotypes of the *LHR* gene at exon 11 among the different cattle ecotypes: The TT, CT and the CC genotypes. The observed genotypic frequencies for *LHR* gene in Kenana were 33.3% for TT, 41.7% for CT and 25% for CC genotypes. In Butana cows, the frequencies were 18.7 for TT, 50% for CT and 31.3% for CC. In Erashy cattle the frequencies were 33.3% for TT, 50% for CT and 16.7% for CC. It was concluded that the allele of *FSHR* gene among the tested animals of Kenana, Butana and Erashy is monomorphic while the *LHR* allele is polymorphic.

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Keywords: Sudanese cattle; RFLP; FSHR; LHR.

1. Introduction

The Sudan indigenous cattle are of zebu type (humped cattle) and are characterized by good adaptation to tropical environment and the pastoral production system.

Molecular biology techniques that employ various molecular markers such as restriction fragment length polymorphism (RFLP) and microsatellites allow the identification of the important genes responsible for the genetic features of interest [1,2,3,4] .

Genetic polymorphism at different genes affecting economic traits have stimulated substantial research interest, because of their impending utilization as an aid to genetic selection or to demarcate evolutionary relationships among different livestock breeds. They were also used to assess the existing biodiversity and differences among cattle breeds which is essential to facilitate the conservation and utilization programs in an effective and meaningful way.

The arrays of new markers have been developed to carry out the genetic variation studies at DNA level [5,6]. Some studies including a few dealing with Sudanese cattle ecotypes were concerned with establishing genetic relationships in cattle populations from Africa, Europe and Asia based on different genetic marker systems[7,8,9]. However the samplesize of Sudanese cattle breeds in these studies did not allow a meaningful estimate of the extent of diversity or relationships among different populations.

FSH and LH receptors are both transmembrane receptors necessary for hormonal functioning during reproduction. They are found in the ovary, testis and uterus. FSH receptor is required by the FSH in the ovary to start and maintain follicular development by binding to its specific receptor (FSH Receptor) on the surface of the granulosa cells. The importance of the identification of DNA polymorphisms in these genes lies in their relation with productive and reproductive performance. They play an important role in ovarian physiology and are valuable for multiple ovulation and embryo transfer. They may allow the selection of females that are carriers of the desired alleles for maximum response to FSH in order to improve reproductive performance. The LHR on granulosa cells of the follicle is essential for LH mediated physiological effects in the final stages of follicular growth, final maturation of the oocytes, ovulation and luteinization of the follicular wall.

The follicle stimulating hormone starts and maintains follicular development by binding to its specific receptor in the surface of the granulosa cells in the ovary [10,11]. This binding allows the activation of the follicle stimulating hormone codifying gene [11]. This gene is located in chromosome 11 and its structure is determined by 10 exons and 11 introns; the first 9 exons include the extracellular domain, whereas exon 11 includes the transmembrane domain [12,11]. The importance of the identification of DNA polymorphism lies in their relation with productive and reproductive genotype[13,1,14]. The existence of allelic variants in FSHR gene reported in cattle [15,4,16,17] indicate that the *FSHR* gene is polymorphic. These changes in the molecular structure of the *FSHR* gene cause desensitization of the FSH receptor in the cell membrane which results in less efficient hormone signal transmission [18,19].

The luteinizing hormone gene influences various activities such as steroidogenesis, follicular growth, oocytes maturation, ovulation and *corpus luteum* formation, which are essential for reproductive function [20]. Acquisition of the luteinizing hormone receptor (*LHR)* on granulosa cells of the dominant follicle is essential to physiological LH-mediated effects on the final stages of follicular growth, final maturation of the oocyte, ovulation and luteinization of the follicular wall. Therefore under physiological conditions the appearance of the *LHR* on the granulosal cells is fundamental for folliculogenesis until ovulation [21,22,23,24].

The objective of the present study was to determine the allelic variants of the *FSHR* and *LHR* genes in three Sudanese indigenous cattle ecotypes (Kenana, Butana and Erashy).

2. Materials and methods

2.1 Sample collection

Three local Sudanese cattle ecotypes Kenana, Butana, and Erashy were included in this study. One hundred and sixteen blood samples from unrelated animals were collected on filter papers (Kenana 34, Butana 32 and Erashy 50). The samples were collected from the homelands of the three ecotypes in the central and eastern parts of Sudan. Kenana cattle are found mainly in Sennar and Blue Nile States spreading in the area between the White and Blue Nile Rivers. This is roughly a triangular area bounded by Sennar, Singa and Kosti towns and lying approximately between latitudes 10º and 13º North longitudes 32º and 34º East. Butana cattle are named after their homeland, the Butana plains of central Sudan which lies between the Nile, Atbara and the Blue Nile Rivers. On the other hand; the Erashy cattle are mostly found in Al-gash area in Gedaref, Kassala and Red sea States and are raised by the Hadandowa tribe.

2.2 Isolation of genomic DNA

DNA was isolated from the blood collected on the FTA papers (Whatman International Ltd, UK.) and this was done by (anon-enzymatic method – phenol precipitation) as described by [25].

2.3 Genotyping

The amplification of *FSHR* and *LHR* was conducted in a similar way to that described by [3].for *FSHR* and [2]. for *LHR* genes. For *FSHR*, the PCR was carried out in the Central Laboratory of the Ministry of Science and Communication with forward primer 5´-CTGCCTCCCTCAAGGTGCCCCTC-3` and reverse primer 5´-AGTTCTTGGCTAAATGTCTTAGGGGG-3`. The forward primer for the *LHR* was 5´-CAAACTGACAGTCCCCCGCTTT -3`and reverse primer 5´-CCTCCGAGCATGACTGGAATGGC3`. Each PCR reaction was done with about 200 ng DNA, in a final reaction volume of 25 µL, containing 10X PCR buffer (20 mMTris-HCl, pH 8.4, 50 mMKCl), $MgCl_2$ (3.5 mM for *LHR*; 2.0 mM for *FSHR*), 0.5 mM of each dNTP (deoxynucleotide triphosphate), 0.4 µM of each of the primers and 1 unit of Taq DNA polymerase. The genotypes were identified through 1.5% agarose gel electrophoresis and by the absence or presence of restriction sites, recognized by the restriction endonucleases *HhaI and AluI* for the *LHR* and *FSHR* genes, respectively. The mixture of PCR products with the enzymes was incubated at 37°C for 3 hours.

2.4 Estimation of allele and genotype frequencies

The genotypic and allelic frequencies for the *FSHR* gene and *LHR* genes were determined by direct counting method according to [26].

To determine, if the population was in Hardy Weinberg equilibrium (HWE) for the *LHR* locus the deviations of the number observed genotypes from the expected genotypes were assessed using the Chi-square test.

3. Results and Discussion

The *ALu* digestion of the 306 bp PCR product at the *FSHR* gene produced only one allele, namely A (243 bp and 63 bp) and indicating the AA genotype (Figure 10).

The 303 bp PCR products for the C/T substitution of *LHR* gene were restricted by the *HhaI* enzyme, which distinguished two alleles (C and T) (Figure 2).

The samples with 155 bp and 148 bp were considered as CC genotypes.

Fragments with 303 bp, 155 bp and 148 bp were considered as CT genotypes and those with 303 bp were considered as TT genotypes. In the *FSHR* gene, all the genotyped cows in Butana, Kenana and Erashy cattle were homozygous for the AA genotype.

3.1 Figures

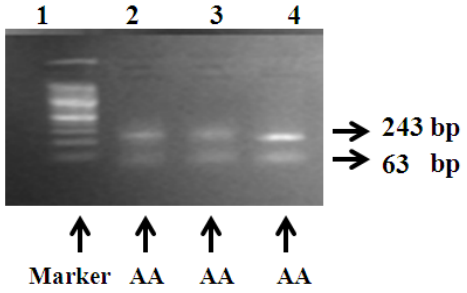

Figure 1: Gel electrophoresis of PCR Products of FSHR gene after digested with *AluI* restriction enzyme.

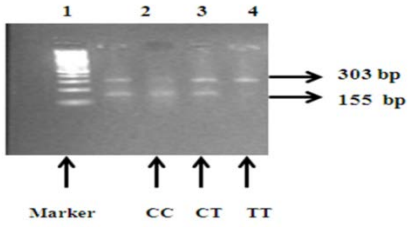

Figure 2: Gel electrophoresis of PCR Products of LHR gene after digested with *HhaI* restriction enzyme.

The genotype frequencies of *LHR* gene for Butana, Kenana and Erashy cows for CC, CT and TT genotypes are presented in Table 1.

Frequencies of allele C and T, for the three breeds were 56.3% and 43.7% for Butana cows, 45.8% and 54.2% for Kenana cows and 41.7% and 58.3% for Erashy cows. There was no significant difference between the three Sudanese cattle breeds regarding the *LHR* genotype frequency (P-value=0.01).

The finding of genotype AA, allele A (243 and 63 bp) in Kenana, Butana and Erashy cattle in the present study is similar to that reported by [4] as allele C (243, and 63) bp in Zebu hybrid cattle in

Brazil.

The finding suggests that the molecular weight could be characteristic of Zebu cattle in South and Central America were imported from India and Spain [5, 29].

Table 1: Genotype frequencies of *LHR* gene in Butana, Kenana and Erashy

Allele frequencies			Genotype frequencies			
Breed	C	T	CC	CT	TT	No of animal
Kenana	45.80%	45.20%	25.00%	41.70%	33.30%	32
Butane	56.30%	43.70%	31.30%	50.00%	18.70%	34
Erashy	41.70%	58.30%	16.70%	50.00%	33.30%	50
Total	48.80%	51.20%	25.00%	47.50%	27.50%	116

The observed genotypic frequencies for *FSHR* gene in Sudanese cattle ecotypes revealed only the presence of the homozygous genotype AA for all ecotypes in this study and the genotype CC which is the predominant genotype of *Bostaurus*was not found in Sudanese zebu cattle. The same trend was also observed in other *Bosindicus* herds and Nelore cattle with high frequencies of AA homozygous genotypes (75%). [3] Reported a high value for genotype AA (49%) though she did not find the genotype CC in the Nelore population as reported by [29].

The high frequency of allele A which characterized Zebu cattle in this study indicates a predominance of *Bosindicu*s genotype in Sudanese local cattle types, Since samples were collected from the homelands of the three different ecotypes, it is likely that the animals sampled were not subjected to cross breeding with *Bostaurus* breeds.

The existence of allelic variants in the *FSHR* gene reported in cattle [15, 17, 1, 4, 16] indicates that the *FSHR* gene is polymorphic. These changes in the molecular structure of the *FSHR* gene cause desensitization of the FSHR in the cell membrane, which results in a less efficient hormone signal transmission [18, 19]. Sudanese cattle are monomorphic in configuration thus expressing this desensitization probably in the form of delayed

puberty and extended length of the postpartum period. These results allow us to conclude that Sudanese cattle may be used for crossbreeding, in order to obtain economically valuable reproductive and productive traits in the crossbreds. Thus, the characterization of the allelic variability of the *FSHR* gene in different cattle breeds allows taking advantage of heterozygosis and selecting individuals that are carriers of specific alleles in loci of reproductive importance [4].

In addition, the *FSHR* gene has 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. [11, 28] reported that the changes in the DNA sequence can affect the activation of *FSHR* gene and they also indicated that the genotype play an important role in ovaraian physiology.

In this study the genotyping of *LHR* gene revealed the presence of TT, CT and CC genotypes with a high frequency of heterozygous genotypes in Butana, Erashy (50%) and Kenana (41.7%). In Brazil Nelore females, different values of heterozygosity ranging from 0.430 and 0.174 were found by [2, 29] respectively.

The characterization of allelic variants of the *LHR* gene for different cattle genotypes contributed to the knowledge of the behavior of this gene under tropical climatic conditions. Further studies need to be carried out to determine the ovarian response to *LH* for each of these allelic variants in cattle, as it would allow the selection of heifers that are carriers of the desired allele for maximum response to *LH* in order to improve the reproductive performance of bovine herds. The heifers identified by the CT/CC haplotype and double heterozygous CT/CG had a higher pregnancy rate of 79% (N=28) and 70% (N=116), respectively. However, the haplotype effect on probability of pregnancy (PP) was confirmed in the composite population studies [16].d. In local Sudanese cattle haplotype CT (47.5%) LHR and haplotype CC (100%) FSHR. The population of Sudanese cattle with regard to these two genes is not in Hardy- Weinberg equilibrium. This might have resulted from continuous natural selection for adaptive alleles and inbreeding. Moreover, the equibrium may have been affected by other systemic factors and strong selection pressures which participated in the formation of the herd [30, 31]. The information about genetic variability and the structure of genetic differences within and between populations make it easier to develop new selection strategies for animal breeding and conservation programs and to define new sources of genetic variation for future generations.

4. Conclusion

It was concluded that the *FSHR* gene among the tested animals of Kenana, Butana and Erashy was monomorphic while the *LHR* gene was polymorphic. This study provides potentially useful information about the genetic structure of the *FSHR* and *LHR* genes which may be used for future breeding and conservation programs.

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