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Genetic variation of CYP19 (Aromatase) gene in Sahiwal cow

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Abstract

Currently, the primary thrust of research in animal genetics is the identification of gene (so-called major genes), which after the expression of quantitative traits markedly. One of the major genes is CYP19 gene which is related to reproduction and production traits in livestock. This study aimed to evaluate the genetic polymorphism within CYP19 gene in Sahiwal cattle using PCR-RFLP technique. Genomic DNA extracted from 60 healthy cows was amplified using primer that were designed from the cattle CYP19 gene sequences. The polymorphism in CYP19 gene was detected using PCR-RFLP method and *PvuII* restriction enzyme. The frequency of genotype AA was found to be 48.33%, AB was 41.66% and BB was 10.0% respectively. The allele A allele was more frequent (0.69) than the allele B (0.31). In population was found deviations from Hardy-Weinberg Equilibrium (HWE).

Keywords: Aromatase cytochrome P450, CYP 19 gene, Genetic polymorphism, PCR-RFLP, Sahiwal cattle

Introduction

Among indigenous breed of cattle, Sahiwal is one of the best milch breed of India [1], producing high milk an average of 2585 kg per lactation [2]. It is comparatively a heavy breed with symmetrical body and loose skin. It is considered as one of the best Zebu milch breed in the tropics with higher disease resistance and heat tolerance properties [3]. Among the indigenous cattle Sahiwal, play an important role in milk production/economy of Rajasthan.

Reproductive performance is an important factor in determining dairy herd profitability. Successful breeding and milk production depend upon fertility performance of female animals, which must be bred at the right time after estrus is observed. The precise information about the incidence and economic losses due to abnormalities of the reproductive tract is not exactly available in India. A good reproductive performance positively affects cattle herd active life and plays an important role in dairy herd economics. Reproduction is influenced by many environmental and genetic components, as are most complex traits. In contrast, long generation interval and the low heritability of reproduction trait can cause limited success in the selection for reproductive traits, such as fertility.

The estrus behavior and productivity in farm animals, are essential regulated by status of ovarian hormones, the important being estrogen. The hormone is essential for the development of dominant follicles in the ovary. A low estrogen level is one of the predominant cause for true anestrus [4]. Most notably high estrogens level is found to be a marker in selection of dominant follicle in bovine [5] buffalo [6]. Aromatase cytochrome P450, product of the CYP19 gene, is the enzyme responsible for estrogen biosynthesis from androgen precursors. The regulation of aromatase expression and maintenance of 17 β -estradiol (E_2) levels is one of the prerequisite for follicular maturation.

In cattle, sheep and human, the CYP19 gene expression in gonads is mainly regulated by most proximal, ovarian promoter, Promoter II (PII). Tissue-specific expression of this gene is regulated by the use of various, spatially separated promoter regions [7]. This results in transcript variants with different 5'-untranslated regions (5'-UTRs) but identical coding sequences.[8] Specific transcript variants and promoter regions have been demonstrated in the tissues of several species.[9] These Characteristics make CYP19 a strong candidate gene for reproductive traits. The bovine CYP19 is mapped on chromosomes 10 [10]. However, the CYP19 gene in indigenous cattle has been less extensively investigated. Little information was available on the genetic polymorphism of CYP19 gene in Sahiwal Cattle.

Therefore, the present study has been designed to analyze the polymorphism in promoter region of CYP19 gene in Sahiwal cattle.

Materials and Methods

A total of 60 animals, belonging Sahiwal cattle's were selected to represent the Cattle population as much as possible based on the available information of phenotypic characteristics. Blood samples were collected from 60 unrelated female animals of Sahiwal cattle on the basis of health records maintained at Livestock Research Station, Kodamdesar, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner were utilized in the present investigation. About 3-5 ml of venous blood was taken from jugular vein in the vacuoner tubes under sterile conditions having EDTA (1mg/ml of blood) from each animal. After collection of blood, the vials were shaken gently to facilitate thorough mixing of blood. The vials were then kept immediately in ice box containing ice and gel cool pack and were transport to the laboratory immediately. After reaching to laboratory, the samples were Kept in deep freeze at -4°C until for further use.

Genomic DNA was extracted from blood through spin column method using Blood Genomic DNA Purification Kit supplied by HiMedia Pvt. Ltd., Mumbai with slight modification. A 405 bp fragment of 5'-flanking region of CYP19 was amplified according to Venselow *et al.* [11]. Briefly the sequences of the forward and reverse primers were 5'-CTCTCGATGAGACAGGCTCC-3' and 5'-ACAATGCTGGGTTCTGGACT-3' respectively. The PCR amplification was carried out in 35 cycles at 94°C for 1Min, 61°C for 40 sec and 72°C for 30 sec. The amplified DNA was digested for 14 hrs at 37°C with 1ul of *PvuII* restriction endonuclease. The digested DNA fragments were resolved by polyacrylamide gel electrophoresis on 8% gel in 1x TBE buffer at 120 V for 1.5 h and visualized under UV light. The allele frequencies were calculated by simple allele counting according to the Hardy-Weinberg equilibrium [12].

Results and Discussion

The 405 bp amplified products of CYP 19 gene were digested with *PvuII* RE enzyme as per the protocol. The *PvuII*/PCR-RFLP assay revealed three types of banding pattern, one of them was of 405 bp (AA genotype); second of 327 and 78 bp (BB genotype) and heterozygous pattern had 405, 327 and 78 bp bands (AB genotype) (Figure 1). This revealed that the Sahiwal Breed of cattle used in the present study were polymorphic in nature with two types of alleles A and B. The AA genotype was the most frequent (48.33%) in all the screened samples, followed by the heterozygote AB (41.66%), whereas the BB genotype was the least frequent (10.0%). The frequency of CYP19/*PvuII* A and B alleles was 0.69 and 0.31, respectively. The selected population of Sahiwal cattle was found in Hardy-Weinberg law Equilibrium.

Jadrzejczak *et al.* [13] reported the no polymorphism in the CYP19 gene in Jersey cattle in contrast to present findings where polymorphism was reported in the CYP 19 gene in Sahiwal cattle. The polymorphic pattern for CYP19 gene in Sahiwal breed of cattle revealed by PCR-RFLP with *PvuII* restriction enzymes may be characteristic for Sahiwal breed and could be used to differentiate it with other breed of goats (Jersey cattle). The polymorphic restriction site can be helpful for further screening of India cattle genome. However, a

definitive conclusion requires a large number of animals to be studied.

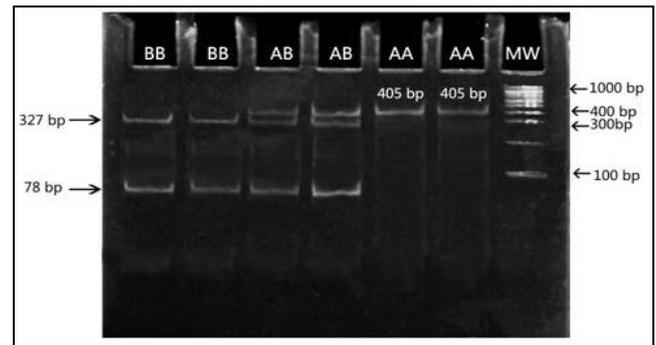


Fig 1: PCR-RFLP pattern obtained after digestion of amplified CYP 19 gene With *Pvu II* in indigenous Sahiwal Cattle
Lane 1-6 (genotypic pattern of Sahiwal Cattle)
Lane 7 MV (Molecular Weight Marker 100 bp ladder)

Conclusion

The genomic DNA extracted from 60 healthy Sahiwal cattle and was amplified using primer that showed the polymorphism in CYP19 gene using PCR-RFLP method and *PvuII* restriction enzyme. It was detected from the blood samples that the A allele was more frequently present than the allele B.

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References

1. Nivsarkar AE, Vij PK, Tantia MS. Animal genetic resources of India: Cattle and Buffalo. ICAR, India. 2000, 382.
2. Tomar AKS, Prasad RB, Bhadula SK. First lactation performance of Holstein, Sahiwal and their half-bred in tarai region of North India. Indian J Anim Res. 1996; 30(2):129-133.
3. Ahmad M, Bukhari A, Awan F, Saeed MN. Livestock and Dairy Development Department Punjab, Lahore, Pakistan. Punjab livestock census, 1, 2000.
4. Hafez ESE, Hafez B. Reproduction in farm animals. 7th ed., Lippincott Williams and Wilkins Publications. 2000, 261-23.
5. Evans ACO, Ireland JLH, Winn ME, Longergan P, Smith GW, Coussens PM *et al.* Identification of genes involved in apoptosis and dominant follicle development during follicular waves in cattle. Biol. Reprod. 2004; 70:1475-1484.
6. Onteru SK, Sharma D, Singh D, Sharma MK. CYP19 (cytochrome P450 aromatase) gene polymorphism in murrh buffalo heifers of different fertility performance. Res Vet Sci. 2009; 86:427-437.
7. Furbass R, Kalbe C, Vanselow J. Tissue-specific expression of the bovine aromatase-encoding gene uses multiple transcriptional start site and alternative first exons. Endocrinology. 1997; 38:2813-2819.
8. Kalbe C, Furbass R, Schwerin M, Vanselow J. Cis-acting elements regulating the placenta-specific promoter of the bovine CYP19 gene. J Mol Endocrinol. 2000; 25:265-273.
9. Bouraima H, Hanoux V, Mittre H, Feral C, Benhalm A,

- Leymarie P. Expression of the rabbit cytochrome P450 aromatase encoding gene uses alternative tissue-specific promoters. *Eur J Biochem.* 2001; 268:4506-12.
10. Furbass R, Kalbe C, Vanselow J. Tissue-specific expression of the bovine aromatase-encoding gene uses multiple transcriptional start site and alternative first exons. *Endocrinology.* 1997; 138:2813-9.
 11. Vanselow J, Zsolnai A, Fesus L, Furbass R, Schwerin M. Placenta specific transcripts of the aromatase encoding gene include different untranslated first exons in sheep and cattle. *Eur J Biochem.* 1999; 265:318-24.
 12. Falconer DS, Mackay FC. Introduction to quantitative genetics. Longman Group Ltd. 1998. ISBN 0-321: 04347-2.
 13. Jedrzejczak M, Szatkowska I, Zych S, Grzesiak W, Piatkowska E, Dybus A. Evaluation of association of the polymorphism in the placenta-specific promotor1.1 of the CYP19 gene in Black-and -White and Jersey cattle with milk production traits. *Arch Tierz Dummerstorf.* 2006; 49(4):311-314.