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DNA polymorphism of Cyp19 (Aromatase) gene in Rathi cattle

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Abstract
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 Rathi using PCR-RFLP Technique. Genomic DNA extracted from 60 health Rathi Cattle and 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 *Pvu*II restriction enzyme. AA (39 %), AB (31.66%), and BB (3.33 %). The allele was more frequent (0.81) than B (0.19). In the population it was found deviations from HWE.

Keywords: Rathi cattle, CYP 19 Gene, Aromatase cytochrome P450, Genetic polymorphism, PCR-RFLP, reproductive gene

Introduction
Rathi breed of cattle is a distinct, relatively unknown breed that possesses good potential with high degree of variability for milk production and has not yet been fully explored for its production potential. Rathi cattle breed were originated from Sahiwal, Red Sindhi, Tharparkar and Dhani breeds with predominance of Sahiwal blood [1]. It shows large variation in coat colour, which varies from light tan to dark brown and blending of blackish tings, also including spotted patterns, a broad fore head, a long tail and is medium sized. There is need to exploit the genetic potential of this breed that is well known for its hardiness to withstand the harsh agro-climatic conditions especially in the drought prone area in arid regions. Genetic improvement of Rathi animals can produce to their full potential even when maintained on dry fodder available in arid regions.

Conventionally selective breeding includes progeny testing and various selection programs, assisted reproductive technologies such as artificial insemination, multiple ovulation, and embryo transfer have been applied and there has been a dramatic improvement in the productivity of animals from the selective breeding of animals. However, traditional breeding techniques in dairy cattle take many years and do not efficiently take into account all sources of genetic variability. Similarly, in sex-limited, low heritable or late-expressed traits, the impact of traditional breeding is limited. The use of molecular markers will help to address the problems associated with traditional selection and thus help to select genetically superior animals.

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 anestrum [2]. Most notably high estrogens level is found to be a marker in selection of dominant follicle in bovine [3] buffalo [4]. 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-estrodiol (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 [5]. This results in

Transcript variants with different 5'-untranslated regions (5'-UTRs) but identical coding sequences [6]. Specific transcript variants and promoter regions have been demonstrated in the tissues of several species [7]. These Characteristics make CYP19 a strong candidate gene for reproductive traits. The bovine CYP19 is mapped on chromosomes 10 [5]. However, the CYP19 gene in indigenous cattle has been less extensively investigated. Little information was available on the genetic polymorphism of CYP19 gene in Rathl Cattle. Therefore, the present study has been designed to analyze the polymorphism in promoter region of CYP19 gene in Rathi Cattle.

Materials and Methods

A Total of 60 animals, belonging Rathi 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 Rathi 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 blood was taken from jugular vein in the vacationer tubes under sterile conditions having EDTA (1mg/ml of blood) from each animal. After collection of blood, the vials were shaken gently to facilitated through 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^{o}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 [8]. 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 ^{o}C for 1Min, 61^{o}C for 40 sec and 72^{O}c for 30 sec. The amplified DNA was digested for 14 hrs. at 37^{0}C with 1ul of *Pvu*II 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 hrs. and visualized under UV light. The allele frequencies were calculated by simple allele counting according to the Hardy-Weinberg equilibrium [9].

Results and Discussion

The 405 bp amplified products of CYP 19 gene were digested with *Pvu*II RE enzyme as per the protocol. The *Pvu*II/PCR-RFLP assay revaled 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 Rathi 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 (65 %) in all the screened samples, followed by the heterozygote AB (31.66%), whereas the BB genotype was the least frequent (3.33%). The frequency of CYP19/*Pvu*II A and B alleles was 0.81 and 0.19, respectively. The selected population of Rathi cattle was found in Hard-Weinberg law Equilibrium.

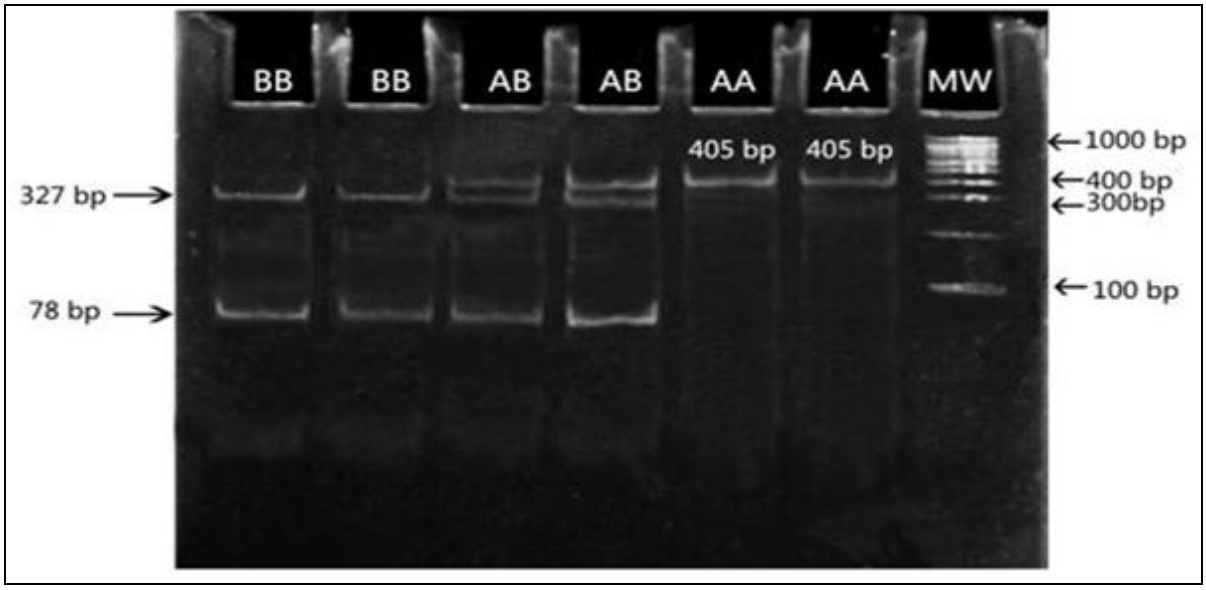

Fig 1: PCR-RFLP pattern obtained after digestion of amplified CYP 19 gene with *Pvu* II in indigenous Rathi Cattle
Lane 1-6 (genotypic pattern of Rathi Cattle)
Lane 7 MV (Molecular Weight Marker 100 bp ladder)

Jadrzejczak *et al.* [10], 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 Rathi cattle. The Polymorphic pattern for CYP19 gene in Rathi breed of cattle revealed by PCR-RFLP with *Pvu*II restriction enzymes may be Characteristic for Rathi breed and could be used to differentiate it with other breed of Cattle (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.

Conclusion

The genomic DNA extracted from 60 healthy Rathi 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 sample that the allele was more frequently present than the allele B.

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