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Monomorphism of luteinizing hormone receptor (LHR) gene in buffaloes by restriction fragment length polymorphism

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Abstract
The productivity of buffaloes is commonly affected due to many inherent disorders. Luteinizing hormone (LH) is a glycoprotein hormone of pituitary origin that regulates gonadal function, including steroidogenesis as well as gametogenesis. The present study was undertaken with 203 Murrah / Graded Murrah buffaloes from different locations to investigate the polymorphism of Luteinizing hormone receptor (*LHR*) gene using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method during June, 2016 to July, 2017. PCR amplification of DNA samples revealed 303 bp product using specific primers and digested with *Hha*I restriction enzyme. All the tested animals revealed monomorphic pattern at 303 bp and genotyped as *TT*, which indicates the fixation of *T* allele and absence of *C* allele. Consequently, we could not perform association studies with reproductive traits.

Keywords: buffaloes, LHR, PCR-RFLP / *Hha*I, monomorphism

1. Introduction
Buffalo has a significant role in the agricultural economy of many developing countries including India by providing milk, meat and draught power. The reproductive efficiency of buffaloes has been influenced by number of genetic, environmental, nutritional and management factors [1]. The reproductive performance of buffaloes are commonly affected due to late maturity, poor expression of estrous, anestrous, inactive ovaries, prolonged postpartum interval, seasonal cyclicity and silent estrous [6, 7, 11]. Many genes are involved in the physiological and endocrine functions of the inherent fertility and account for the genetic association of reproductivity and growth, milk and overall productivity [4].
Luteinizing hormone (LH) is found to be an important hormone directly linked with reproductive functions. LH is responsible for follicular wall rupture, ovulation and stimulates corpus luteum to produce progesterone in females [13]. LH composed of an alpha and beta sub unit, stimulates the interstitial cells of both ovary and testis and also essential for estrogen secretion. The expression of the Luteinizing hormone receptor (*LHR*) in the ovary is induced by follicle-stimulating hormone, estrogen, and growth factors in granulosa cells of the preovulatory follicles.
Any variation of nucleotide sequences in *LHR* genes might directly or indirectly affect reproductive traits. Therefore, *LHR* genes are proposed as a candidate gene for selection of reproductive buffaloes [10]. Recently this polymorphism has been studied in various exotic and Indian cattle breeds, but few in buffaloes [8, 12, 14]. Till date no *LHR* / *HhaI* polymorphism has not been made in Indian buffaloes. Hence, the present study was undertaken to investigate the status of *LHR* gene polymorphism using PCR-RFLP assay in Murrah / Graded Murrah buffaloes.

2. Materials and Methods
A total of 203 Murrah / Graded Murrah female buffaloes maintained at government institutional farms in Tamil Nadu (73) and Andhra Pradesh (86) as well as farmers herds in Namakkal district of Tamil Nadu (n=44) were utilized in the present investigation. Genomic DNA was extracted from blood samples using slightly modified high salt method [5]. A 303 bp fragment of exon 11 of *LHR* gene was amplified using specific forward and reverse primers were 5'- CAA ACT GAC AGT CCC CCG CTT T -3 and 5'- CCT CCG AGC ATG ACT GGA ATG GC -3' respectively [9].

The cycle conditions included an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min and extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR product was checked on 1.0 percent agarose gel electrophoresis in 1x TAE buffer after staining with ethidium bromide (EtBr) and visualized under UV light. The restriction digestion was carried out at 37 °C for overnight in a total volume of 15µl containing 7.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 Units *Hha*I enzyme (Takara Bio USA, Inc.). Digested samples (10 µl) were separated on 2 percent agarose gel containing ethidium bromide at 2 V/cm for 1 hr to determine the genotypes. The gels were visualised and the images were documented in a gel documentation system (Bio-Rad Gel Doc™).

3. Results and Discussion

The amplified PCR products of *LHR* gene (exon 11) was run in 1.0 percent agarose gel and visualized at 303 bp. In the present study, all the digested PCR products of *LHR* / *Hha*I were found monomorphic in nature. The study revealed an uncut banding pattern (*TT* genotype) at the position of 303 bp (Fig. 1). We could not identify any animal with homozygote *CC* (155 and 148 bp) and heterozygote *CT* (303, 155 and 148 bp) genotypes. Similar findings were reported in Egyptain buffaloes [9, 11] and Murrah buffaloes [1]. In contrast to our investigation, *TT*, *CT* and *CC* genotypes were also detected in European- Zebu composite bovine with the genotypic frequencies of 5, 54 and 41 percent respectively [2]. In addition, three genotypes (*TT*, *CT* and CC) were detected in *Bos tarus* x *Bos indicus* beef composite population [3].

In present study, the *CC* and *CT* genotypes were found absent, Similarly, *CC* and *CT* genotypic frequencies were found as zero in buffaloes [9, 11]. Similar to present study monomorphic condition was reported in Indian Murrah/ Graded Murrah buffaloes [1]. The frequency of *T* allele was 1.0 and for *C* allele was 0.0 in the screened buffalo population. Similar allelic frequencies were reported in buffaloes [9, 11]. In contrast, the allelic frequencies of *C* and *T* allele were 45.80 and 45.20 percent for Kenana cows, 56.30 and 43.70 percent for Butana cows and 41.70 and 58.30 percent for Erashy cow respectively [8]. It's indicates, fixation of *T* allele in studied buffalo population. We could not perform association study with reproductive traits of buffaloes in the present study, because all the screened animals were found monomorphic for *LHR* / *Hha*I locus.

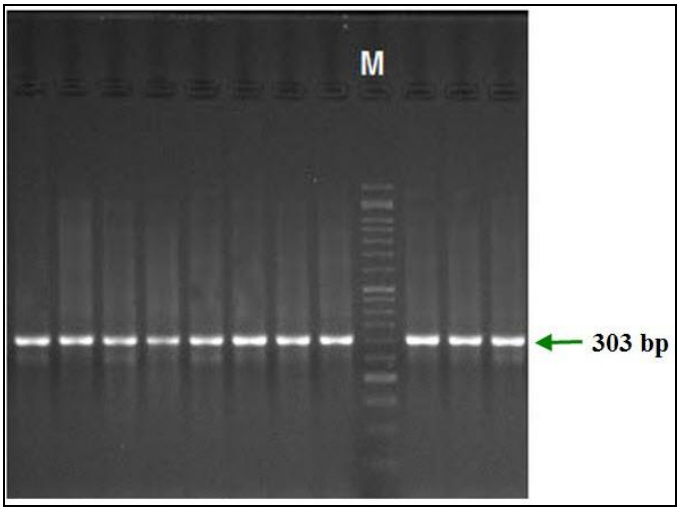

Fig 1: Monomorphic pattern of *LHR* / *Hha*I gene in Murrah and Graded Murrah buffaloes. (M -50 bp DNA Marker)

3.1 Statistical analysis

The detection of polymorphism in buffaloes for *LHR* / *Hha*I gene was found as a monomorphic condition and hence, association studies were not carried out with reproductive traits.

4. Conclusion

In present study, the status of *LHR* gene polymorphism was investigated using PCR-RFLP assay in Murrah / Graded Murrah buffaloes and found as monomorphic pattern in the screened population. Consequently, we could not establish any association between genotypes and reproductive traits because these buffaloes were found homozygous (*TT*) for *LHR* / *Hha*I locus. But the study with more number of animals may lead to genetic variation and that can be used for selection of reproductive animals.

5. References

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