Original Research

Detection of Follicle Stimulating Hormone Receptor Gene Polymorphism in Murrah and Graded Murrah Buffaloes

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Rec. Date:	Nov 13, 2018 06:00
Accept Date:	Apr 20, 2019 16:51
DOI	10.5455/ijlr.20181113060005

Abstract

A study was undertaken in 203 Murrah and graded Murrah buffaloes reared under organised farm and farmers field conditions in Tamil Nadu and Andhra Pradesh to study the polymorphism of FSHR gene by PCR- RFLP method and to analyse the possible associations of these gene variants with age at first calving and calving intervals. The PCR amplification carried out with specific forward (F - 5'- CTG CCT CCC TCA AGG TGC CCC TC -3') and reverse (R – 5'- AGT TCT TGG CTA AAT GTC TTA GGG GG -3) primers. The 306 bp amplified PCR products of FSHR (exon 10) gene was genotyped based on RFLP with AluI restriction enzymes and all the tested animals were monomorphic and showed unique banding pattern which indicated the fixation of C allele in buffaloes.

Key words: Polymorphism, *FSHR*/*Alu*I, PCR – RFLP, Buffalo

How to cite: Kathiravan, R. S., Chitra, R., Murli, N., & Arthanarieswaran, M. (2019). Detection of Follicle Stimulating Hormone Receptor Gene Polymorphism in Murrah and Graded Murrah Buffaloes. International Journal of Livestock Research, 9(7), 143-147. doi: 10.5455/ijlr.20181113060005

Introduction

As per 19th Livestock Census, the total buffalo in the country is 108.7 million numbers and has increased by 3.19 per cent as compared to 18th Livestock Census. Due to late maturity, poor expression of estrous, anestrous, inactive ovaries, prolonged postpartum interval, seasonal cyclicity and silent estrous, buffalo reproductive efficiency was declined (Mishra, 1997; Minji *et al*., 2008; Sosa *et al*., 2016). Animals reared under similar environmental and management condition also have reproductive problems, that indicating genetic factors may also have a key role in animal reproductive efficiency (Kumar *et al*., 2014).

Buffaloes in tropical environment are seasonal breeding animals and their reproductive efficiency is negatively affected by increasing day-length, which consequently influences production. The reproductive performance of buffaloes are affected by many hormones (estrogen, melatonin, follicle-stimulating hormone, luteinizing hormone, progesterone, prolactin, cortisol, etc.) coupled with their respective receptors. Follicle stimulating hormone (FSH) is secreted by anterior pituitary gland and is essential for follicle growth, development, differentiation, triggering the maturation and ovulation of ovarian follicles (Segaloff and Ascoli, 1993; Themmen and Hutaniemi, 2000; Yi *et al*., 2012; Chu *et al*., 2012;).

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. This binding allows the activation of the FSHR codifying gene. Follicle Stimulating Hormone Receptor gene (FSHR) is large and is composed of 10 exons and 9 introns (Simoni *et al*., 1997). The existence of allelic variation in FSHR gene was reported in cattle and these changes in the molecular structure of this gene cause desensitization of the FSH receptors in the cell membrane which results in a less efficient hormone signal (Simoni *et al*., 1997; Jiang *et al*., 2012). A candidate gene approach has been already successfully applied to identify several DNA markers associated with production traits in livestock (Rothschild and Soller, 1997). Recently, investigators and breeders focus on marker-assisted selection (MAS) and genome analysis. MAS may increase annual rate of genetic gain in livestock by 15 to 30 per cent without increasing the risk involved in breeding schemes (Ge *et al*., 2001).

Few studies on polymorphism has been reported in Indian buffaloes. Hence, a detailed study on polymorphism of follicle-stimulating hormone receptor (*FSHR*) gene has been made by PCR-RFLP to identify the candidate gene for reproductive traits in Murrah / graded Murrah buffaloes.

Materials and Methods

Samples and DNA Extraction

A total of 203 Murrah / graded Murrah buffaloes were included in the study (Table 1).

Table 1: Details on number of blood samples collected from Murrah /graded Murrah buffaloes at different location

S. No.	Location of Farm	No. of Blood Samples
1	Post Graduate Research Institute in Animal Science (TANUVAS), Kattupakkam, Tamil Nadu.	34
2	Buffalo Research Station, Venkataramanna Gudem, S.V.V.U, West Godavari District, Andhra Pradesh	86
3	Saraswathi Krishi Vigyan Kendra, Karur district, Tamil Nadu.	19
4	Central Cattle Breeding Farm, Alamadhi, Chennai, Tamil Nadu.	20
5	Farmers herd, Namakkal, Tamil Nadu	44
Total		203

DOI 10.5455/ijlr.20181113060005

Blood samples were collected from jugular vein aseptically and brought in ice to the laboratory and stored at -20°C till processed. Genomic DNA from whole blood was extracted by using high salt method with minor modifications (Miller *et al*., 1988). The quality, purity and concentration of isolated DNA was checked by Nanodrop and agarose gel electrophoresis.

PCR – RFLP Method

The PCR primers used were i.e., F - 5'-CTG CCT CCC TCA AGG TGC CCC TC-3' and R– 5'-AGT TCT TGG CTA AAT GTC TTA GGG GG-3 to amplify a 306 bp PCR fragment harboring parts of exon 10 of the FSHR gene (Othman and Abdel-Samad, 2013).The cycle conditions included an initial denaturation at 94°C for 5min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for1 min and a final extension at 72°C for 10 min. The PCR product was checked on one percent agarose gel electrophoresis in 1x TAE buffer after staining with ethidium bromide (EtBr) and visualized under UV light. The PCR products were digested with 10 U of *Alu*I restriction enzyme (Takara Bio USA, Inc.) at 37°C for overnight or 12 hours. The restriction fragments were subjected to electrophoresis in two per cent agarose gel in 1 X TAE buffer containing ethidium bromide at 2 V/cm for one hour to determine the genotypes. The gels were examined under UV light and the images were documented (Bio-Rad Gel Doc™).

Results and Discussion

The isolated DNA was quantified and checked for quality in Nanodrop. The mean yield of DNA isolated was 450.88 ng/µl. A region of 306bp*FSHR* gene in Murrah / graded Murrah buffaloes were amplified (Fig. 1). The PCR product of *FSHR* (exon 10) gene digested by *Alu*I restriction enzyme showed monomorphic condition.

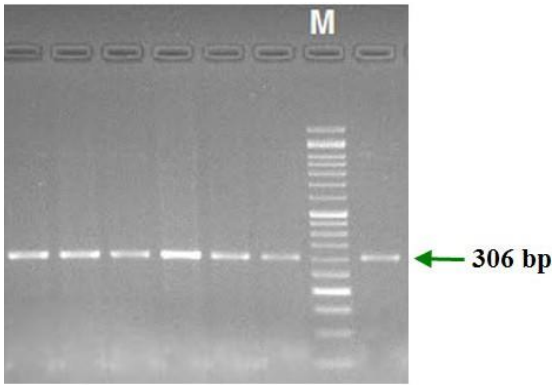

Fig. 1: PCR products of *FSHR* genes in Murrah and graded Murrah buffaloes

Bands of 243 and 63 bp were observed in all the samples corresponding to the CC genotype (Fig. 2) and representing the presence of C allele in studied population. Similar genotype was observed previously by Othman and Abdel-Samad (2013) and Sosa *et al*. (2015) in Egyptian buffaloes from Egypt. The C allele

 DOI 10.5455/ijlr.20181113060005

alone present in the studied Murrah population by PCR-RFLP showed a clear evidence of fixation of C allele in Murrah / graded Murrah buffaloes. But PCR - SSCP study of FSHR gene by Ahmed *et al*. (2011) showed polymorphism with three distinct patterns.

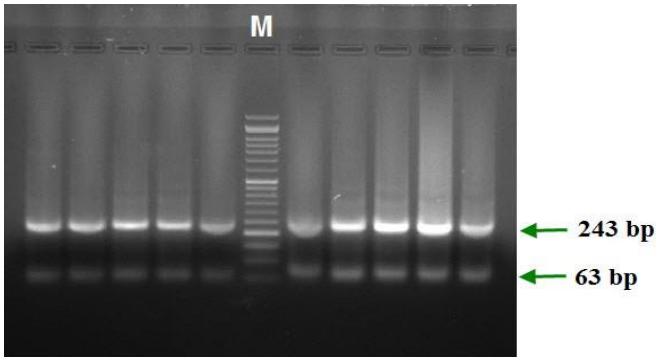

Fig. 2: RFLP patterns of *FSHR* / *Alu*I gene in Murrah and graded Murrah buffaloes

The genotypic frequency of genotype CC was 100% and the allelic frequency of allele C was 1.0 and for allele T was 0.0 in screened animals. Similarly, Othman and Abdel-Samad, (2013) and Sosa *et al.* (2016) also found the frequency of CC genotypes as 100% in Egyptain buffaloes. Contrast to our study, three different genotypes were noticed in other livestock (cattle and sheep) for FSHR gene (Marson *et al*., 2005; Marson *et al.*, 2008; Chu *et al.*, 2012; Yi *et al*., 2012). Since the allele is monomorphic for *FSHR* / *Alu*I locus, we could not establish any association between genotypes and reproductive traits in the present investigation.

Conclusion

In the present investigation, the PCR product (306 bp) of exon 10 of *FSHR* gene was genotyped by RFLP with *Alu*I restriction enzymes and all the screened animals were found to be a monomorphic in nature, which indicated the fixation of C allele in Murrah / graded Murrah buffaloes. Investigation of this gene for polymorphism in other Indian buffaloes is needed to identify suitable genetic marker for this gene to utilize in Marker Assisted Selection to improve the reproduction performance in Indian buffaloes.

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DOI 10.5455/ijlr.20181113060005