Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(2): 207-210
© 2020 JEZS
Received: 28-01-2020
Accepted: 25-02-2020

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Identification of genetic polymorphism of Fec B gene in cattle

A Sudharshan and BR Yadav

Abstract
Fecundity is the potential reproductive capacity of an individual or population. Identification of the genes affecting twinning rate will increase the basic understanding of the reproductive process and may also be used to improve reproductive efficiency in cattle. However, so far fecundity genes influencing multiple births have not been studied extensively in cattle. An investigation was carried out for identification of Fec B gene (BMPR 1B) polymorphism in cattle, with comparison of DNA fragment between dams bearing twin and singleton. The animals belonged to six breeds *viz*. pure (Sahiwal and Hariana) and three crossbreeds (Karan Swiss, Karan Fries, Holstein Friesian cross) besides other cross breeds of unknown lineage selected from field. The study involved analysis of genome related to twinning using molecular marker (PCR-RFLP) on Fec B gene. Molecular study was carried out using one primer on the DNA of 26 animals out of which 20 were experimental dams, 6 were control dams. PCR-RFLP analysis of the BMPR-1B gene using *Ava*II revealed monomorphic band pattern of the wild-type allele in the sampled animals. The study revealed monomorphic pattern of the Fec B gene without influencing twinning trait in cattle.

Keywords: Fecundity, polymorphism, Fec B gene, cattle

Introduction
Fecundity, derived from the word fecund, generally refers to the ability to reproduce. In demography [10, 11] fecundity is the potential reproductive capacity of an individual or population. Cattle are the most common type of large domesticated ungulates. Different breeds have been developed for various purposes, *viz*. meat (beef and veal), milk and other products, and as draft animals (oxen/bullocks) (pulling carts, plows and the like). Other products include leather and dung for manure or fuel. In some countries, such as India, cattle are considered sacred and are raised mainly as dairy animal. Ovulation is the process by which a mature ovarian follicle ruptures and discharges an ovum (also known as an oocyte, female gamete, or casually, an egg). Multiple ovulations in mammals are a complex trait influenced by genetic and environmental factors. Many mammals, such as humans, cattle, sheep, goats, and deer, normally have an ovulation rate of one egg per cycle, whereas other mammals, such as rats, mice, hamsters, cats, dogs, and pigs, have ovulation rates that vary between 4 - 15 eggs per cycle [18] . Cattle are primarily monovulatory, and the frequency of fraternal twin or multiple births is low. However, the frequency of twinning can be increased by hormone therapy [9], embryo transfer [1], or long-term genetic selection [10, 14]. Twins in cattle are not common but about 1 set in every 4000 births has been reported. Sheep has been considered as a model species for studying genes involved in mechanisms controlling ovulation rate. Genetic variation associated with ovulation rate in sheep has been widely documented and show substantial evidences [3]. Mutation studies in different prolific sheep breeds have shown that the transforming growth factor beta super family ligands *viz*. the growth differentiation factor 9 (GDF9/FecG), bone morphogenetic protein 15 (BMP15/FecX) and associated type I receptors, bone morphogenetic protein receptor (BMPR-1B/FecB), are major determinant of ovulation rate and consequent increase in litter size. In cattle, twinning may have both desirable and undesirable effects. The major contribution of twinning would be an increased calf crop in both beef and dairy cattle [2]; in the latter an increase in the number of offspring of genetically superior females may also be important [13].
Rate of reproduction has a major impact on life cycle, costs of production of different animal species and on their competitiveness for different types of production resources. Reproductive performance is a major determinant of profitability for cattle producers.

So far the genetic factors that affect reproduction are not well understood, and identifying these factors could lead to substantial improvements in these traits, primarily by genetic selection but also with Pharmaceuticals to enhance reproduction. Identification of the genes affecting ovulation rate and twinning rate will increase the basic understanding of the reproductive process and may also be used to improve reproductive efficiency in cattle. Though, there exists conservation in functional genes in mammalian species and a high number of heterologous primers and probes unravel genes across the species. Application of molecular markers will help in identifying the genes influencing fecundity and twinning in cattle. The present investigation has been an attempt to identify the Fec B gene using PCR-RFLP.

Material and Methods

The animals investigated in the present study were from the cattle yard of National Dairy Research Institute, Karnal, some dams were selected from the field in Haryana and Punjab, information for which were gathered through communication in newspaper. The details of animal selected given in (Table 1) Experimental animals were selected based on criteria cattle dams which gave birth to twins at least once in their lifetime and twin born individuals one or both members of twin pair available. Control animals were selected based on cattle dams which gave birth to singletons in all calving. Blood samples were collected from the dams and twin born progenies. Aseptically 10-20 ml of blood per animal was collected from jugular vein in separate sterile tubes containing Acid Citrate Dextrose (ACD) @ 1 ml / 6ml blood as an anticoagulant. DNA was isolated from the blood samples using routine standard phenol-chloroform extraction method [4] with some modifications [21].

Table 1: The details of the animals selected

Breed	Dam	Control Dams
Sahiwal	1	3
Hariana	2	
Karan Fries	8	3
Karan Swiss	2	
Holstein Friesian X	5	
Crossbred	2	
Total	20	+ 6 = 26

PCR conditions

The Fec B polymorphism was carried out by forced PCR-RFLP technique using F-(5'-CCAGAGGACAATAGCAAAGCAAA-3') and R-(5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3') [5]. The reverse primer was designed [5] to introduce a point mutation in the amplified products that creates AvaII restriction site in the DNA with FecB mutation. Then the AvaII enzyme would cleave the DNA with FecB mutation that would produce two fragments of 160 bp and 30 bp while the wild type remains uncleaved. The PCR reaction was set to a volume of 25 µl.

The amplification reaction conditions were as follows; initial denaturation at 95 ºC for 120 s, followed by 95 ºC for 30 s, 60 ºC for 30 s, 72 ºC for 30 s for 34 cycles and final extension at 72 ºC for 5 Min. The 190-base pair (bp) product was then digested using AvaII, and the resulting products were resolved on a 2.5% agarose gel horizontal electrophoresis and visualized by ethidium bromide staining. The restriction digested gene fragments were visualized on UV Transilluminator and photographed with gel documentation system. The DNA fragments were analyzed by visual comparison of band patterns in the sampled animals.

Results and Discussion

Several studies have documented the effect of multiple births on the productive and reproductive performance of cows. Multiple births reduce the reproductive efficiency of cows by increasing difficulties during the pregnancy and calving periods. Cows calving twins produce less milk during the subsequent lactation compared with cows calving singletons [20]. Even though the reason for twinning in cattle is not known it is necessary to find the Fec B polymorphism causing multiple births in few sheep breeds may be responsible for twins in cattle. Amplification of exon six of the BMPR-1B gene by PCR produced amplicon size 190bp using known primer [5] in dams both experimental (20) as well as control (6). PCR products were resolved in 1.5% agarose gel electrophoresis for confirmation. The PCR products was digested with *Ava*II restriction enzyme at 37 °C for 5 h and the resulting products were separated on a 2.5% agarose gel, visualized with ethidium bromide and detected by UV transilluminator.

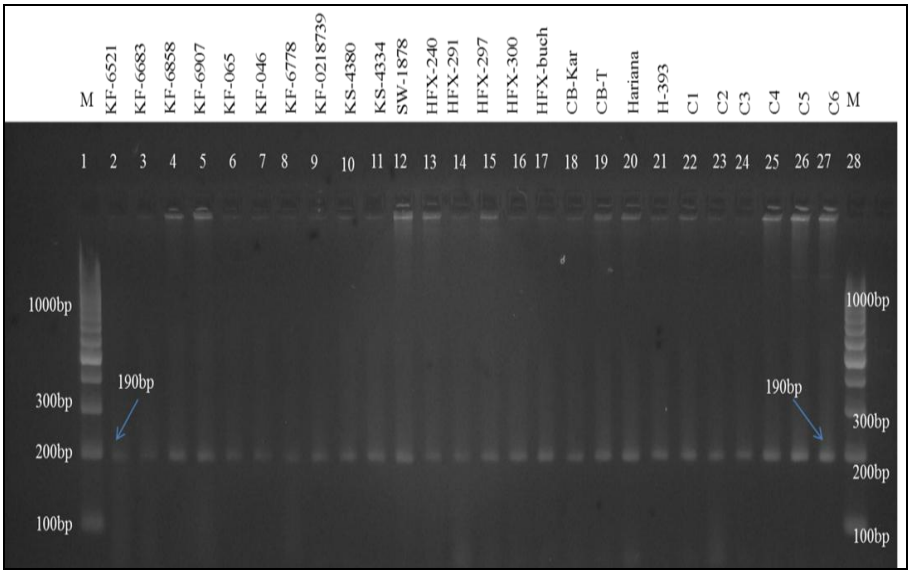

Fig 1: RFLP product of Fec gene primer digested with *Ava*II RE revealed by 2.5% agarose gel in cattle dams. Lane 1, 28: 100bp DNA ladder, Lane 2-27: RFLP product of 190 bp

The FecB amplicon of 190bp size in cattle dams subjected to *Ava*II restriction enzyme digestion did not reveal any cutting site as shown in (Fig. 1), only the wild type allele + was present in monomorphic state. PCR-FRFLP pattern identification of the restriction fragments was done according to the PCR-FRFLP patterns of the BMPR-1B gene reported in sheep. PCR FRFLP analysis of the BMPR-1B gene revealed that the Fec B (Booroola) mutation was absent in all the dams analyzed and showed monomorphic pattern of the wild type allele Fec B+. In livestock, some DNA markers have been found to be associated with variations in reproduction traits, such as polymorphisms in the estrogen receptor gene with litter size in pigs and Q249R mutation in the *BMPR-IB* gene with ovulation rate in sheep. Current knowledge of major genes for prolificacy in sheep falls into three categories: (1) genes where the mutation has been identified and DNA testing is available; (2) genes where the mode of inheritance has been described but the mutation has not been identified; and (3) putative genes where there is evidence of apparent genetic segregation but there are insufficient records to ascertain the mode of inheritance. The Fec B mutation was first confirmed in Booroola Merino by three different groups of scientists (Mulsant P, Souza C.J.H, and Wilson, T) from Australia [19, 22, 24, 5]. Davis reported that the Fec B mutation was present at a high frequency in the Garole sheep suggested that FecB is fixed (all sheep homozygous carriers of the gene) in some Garole populations. Garole ewes initially sampled were homozygous (Fec B^B/Fec B^B) for the Booroola gene with 160bp band size. Javanese breed from Indonesia also revealed the similar banding pattern as Garole after digestion with *Ava*II the FecB homozygous carriers (BB) animals had 160 bp band, heterozygous carriers (B+) animals had 160 and 190 bp, and the homozygous noncarriers (++) animals had 190 bp band pattern. **A**bsence of Fec B mutation, have been observed in different sheep breeds of world like Loa, Chios, D'Man., Barbados Blackbelly [7] and of India like Malpura and Madgyal sheep[15,16]. In contrast to this result, Fec B is currently known to be present in Booroola Merino, Garole, Javanese, Han, Hu [6], Kendrapada [17] sheep without an intentional introgression. Australian Booroola Merino has largely contributed to FecB allele introgression into many breeds which is now identified in 48 breeds and composites in 19 countries [7]. In India, FecB mutation has been identified in three breeds *viz*., Garole, Kendrapada and Nilagiri sheep [6, 17, 23]. Mutation study has shown that gene mutations in the highly conserved domain of the bone morphogenetic protein receptor IB (BMPR-IB) gene located on ovine chromosome 6 is fully associated with the hyperprolific phenotype of Booroola Merino ewes. The amino acid substitution at position 249 of the BMPR-IB protein impairs its inhibitory effect on granulose cell steroidogenesis leading to an advanced maturation of follicles [19, 22, 24]. Sheep chromosomes 6 and X correspond to bovine chromosomes 6 and X, respectively [8]. As yet, however, there are no indications that chromosomes 6 and X could be connected with twinning or ovulation rates in cattle. The presence of same pattern combination in the present study in comparison to the some sheep breeds indicated that the sixth exon of the BMPR-1B gene is monomorphic in cattle whereas in case of some sheep breeds it is highly polymorphic. Mutation of Fec B gene associated with twinning in sheep was not found in cattle.

Conclusion

From the study conclusion emerged that the fecundity genes identified in sheep causing high litter size did not reveal relation with twinning trait in cattle. The FecB mutation was absent in cattle, only the wild type alleles was present. Absence of this gene mutation provides evidence that the Booroola gene has no effect on prolificacy of cattle.

Acknowledgements

I sincerely acknowledge ICAR-NDRI, Karnal, to provide facilities. Also to R.K Tong, Technical officer, Livestock genome analysis laboratory, ICAR-NDRI, Karnal, for laboratory assistance. This investigation is a part of the M.V.Sc (Animal Genetics & Breeding) degree program of first author from NDRI, Karnal.

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