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DETECTION OF Y CHROMOSOME OF BOVINE USING TESTIS SPECIFIC PROTEIN AND AMELOGENIN GENES

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ABSTRACT. A total of thirty-eight Mafriwal cattle were selected from a local cattle herd of a government cattle farm; of which 36 animals were sub-fertile Mafriwal female dams and two bulls which were considered as control animals (one male Mafriwal and one male Jersey). Two markers were used in the detection of Y chromosome in the sub-fertile female animal which are testis specific proteins Y-encoded (TSPY) and amelogenin (AMLX/AMLY) genes. The genes were amplified using PCR. The DNA bands from a normal male for TSPY gene size was approximately 260 bp while AMLX/AMLY gene were approximately 341 and 467 bp. The examination of all samples showed that the sub-fertile cow revealed only 467 bp while three fragments were detected in the control group; 260 bp (testis specific protein, Y-encoded gene), 341 and 467 bp (Amelogenin gene). The results showed that the sex chromosome anomalies associated with Y chromosome did not occur in this group. These two sex markers can be used for the diagnosis of Y chromosome abnormality in a sub-fertile cow through polymerase chain reaction which is a rapid and reliable method for use in breeding herds.

Keywords: Mafriwal cattle, testis specific protein gene, amelogenin gene, sub-fertile female, PCR

INTRODUCTION

There are three fertility conditions that can occur in cattle namely fertile, subfertile and infertile. Fertile conditions are where a cow which meets the fertility criteria established for the herd, which is, ideally it should be calving every 12 months. A condition of being less than normally fertile though still capable of effecting fertilization is said to be subfertile. While infertile is referred as a sterile cow, also known as incapable of getting pregnant.

In the cattle industry, calf production in female cows is one of the most important traits producers are looking for in terms of economic stability in their herds. The selection of heifers that conceive in their first calving season and then produce a calf every year thereafter are most profitable. The heifers that fail repeatedly to get pregnant are a major concern faced by farmers in all countries. The reduction in number of pregnancies may lead to decreasing number of calves produced and marketed for the beef sector and milk production for the dairy sector. The reproductive efficiency is thus commonly related to fertility problems occurring in the livestock industry. Determining the existence of Y chromosome fragments in a heifer's DNA can lead to better heifer replacement decisions, both for beef and dairy herds.

Typically, females inherit an X chromosome from each parent (XX) and males inherit an X and a Y (XY). Most infertility cases in cattle reported are associated with chromosome anomaly resulting in Freemartins Syndrome, that is, a female cow that was a twin *in utero* with a male cow and had been receiving Y chromosome influences through blood transfer from the male cow throughout the gestation. Other possible abnormalities are Klinefelter Syndrome (61,XXY), Turner Syndrome (59,X) and Trisomy (61,XXX). Cattle with chromosome abnormalities phenotypically look normal but there are fertility issues. The presence of Y chromosome in females, frequently results in fertility problems (Shahrum *et al.,* 1995).

Using molecular techniques, the determination of an animal's sex as well as diagnosing the X/Y chromosome occurrence can be done by using the PCR method which is faster and more precise compared to conventional karyotyping which may be more accurate but time consuming and requires a trained cytogeneticist. The PCR amplification method can be done using X/Y linked chromosomal sequence specific primers of specific markers such as testis specific protein Y encoded (TSPY), zinc finger protein (Zfy/Zfx), amelogenin genes (AMLX/AMLY), as well as other Y-specific markers (Shaharum *et al.,* 1995; Mukhopadhyay *et al.,* 2011).

The selection of a genetic marker in the DNA region associated with reproductive traits is challenging due to its low heritability (McDaneld *et al.,* 2011). However, it is possible to perform this using high density single nucleotides polymorphism arrays. In this study, the candidate genes used were testis specific protein (TSPY) coded by the Y-chromosome which is a Y-specific gene (Affara *et al.,* 1996) and amelogenin gene which is homologous but non-identical and is also located on the X and Y chromosome and can serve as the internal control for the successful amplification using the same sets of primers (Pierce *et al.,* 2000; Lemos *et al.,* 2005). Testis specific protein Y-encoded and amelogenin genes have previously been used to determine the sex

of mammalian species including humans and cattle (Lemos *et al.,* 2005). In the bull, testis specific protein Y-encoded revealed a DNA fragment of approximately 260 bp (Lemos *et al.,* 2005) while amelogenin gene was approximately 467 and 341 bp (Chen *et al,.* 1999), respectively.

This preliminary study was conducted to determine the presence of Y chromosome in a sub-fertile Mafriwal cow using Y-specific markers, testis specific protein Y-encoded and amelogenin genes. This animal, which kept in isolation as it was a repeat breeder having difficulty in conceiving following the routine breeding programmes. It was suspected to have a genetic abnormality in the sex chromosome; that is associated with the Y chromosome. This marker could be used as the screening tool for early detection of Y chromosome in a sub-fertile cow through PCR which is a rapid and reliable method for routine use in farms.

MATERIALS AND METHODS

Sampel Selection

A peripheral blood sample was collected from 36 sub-fertile Mafriwal cows. The control group consisted of two normal bulls (I/D No: I63 4737, Breed: Mafriwal) and (I/D. No: IJZ 4603, Breed: Jersey) that were proven sires. The Mafriwal cattle were sourced from PTH Air Hitam, a govenment cattle breeding farm, while the male Jersey bull was from the Institut Biodiversiti Veterinar Kebangsaan farm. The male had the semen collected into EDTA tubes and kept in -20 °C before further processing was done.

Molecular Techniques Performed

The genomic DNA was isolated by using commercial DNA Extraction Kit (Genedirex). The extracted DNA was appropriately labelled and stored at -20 °C for analysis. The oligonucleotide sequence of the primers of TSPY gene used by Lemos *et al.* (2005) was: Forward: 5'-CCCGCACCTTCCAAGTTGTG-3' and Reverse: 5'-AACCTCCACCTCCTCCA CGATG-3'. The oligonucleotide sequence of the primers of amelogenin gene was used by Chen *et al.* (1999) on X- and Y-chromosome of bovine was: Forward: 5'-AAATTCTCTCACA GTCCAAG-3' and Reverse: 5'-CAACAGGTA ATTTTCCTTTAG-3'. The PCR reaction volume of 25 µl contained 1× Toptaq Master Mix (Qiagen, USA), 0.5 µM of each primer and 1.0 µl of DNA templates. PCR was carried out using Applied Biosystem thermocycler with the following conditions: initial denaturation at 94 °C for 3 mins, followed by 32 cycles, denaturation at 94 °C for 30 s, annealing at 63 °C for 40 s (TSPY gene) while 54 °C for 40 s (AML gene) and extension at 72 °C for 60 s. A final extension at 72 °C for 5 mins at the end of the amplification cycles was included. This test was repeated three times for each sample to gain more reliable results.

Gel Electrophoresis and Analysis

The PCR product obtained was analysed by MetaPhor (Lonza) agarose gel 3% in 1× TBE buffer for 2 hours at 70 volt. The gels were stained with Fluorosafe (1st Base) and visualized under UV light on a transilluminator. The PCR fragment size was determined using software GeneTools Ver. 3.08 (Syngene).

RESULTS AND DISCUSSION

The results of a PCR amplification of testis specific protein Y-encoded (TSPY) gene from normal bulls (control group) and sub-fertile cows using primers introduced by Lemos *et al.* (2005) and Chen *et al.* (1999) are shown in Figure 1. The examination of all samples showed that sub-fertile cows revealed only 467 bp whereas three fragments were detected in the control group: 260 bp (testis specific protein, Y-encoded gene), 341 bp and 467 bp (amelogenin gene). This result indicates that all the sub-fertile cows do not possess any Y chromosome. The PCR using TSPY specific primers was specific since all of the male samples were TSPY positive while all the female samples were negative.

Many studies associated with sexing diagnosis favoured the use of testis specific protein Y-encoded (TSPY) as a candidate gene because this gene is very specific to male mammals and it can be detected at low concentrations of DNA (less than 1 pg/µl) (Lemos *et al.,* 2005; Arash *et al.,* 2011) while amelogenin gene could be used as a control set of primers because it is homologous but not identical which exists in both sex chromosomes. Arash *et al.* (2011) reported that TSPY and amelogenin genes could be used as candidate genes for non-invasive bovine fetal DNA diagnosis which is considered a successful assay to determine the gender of the offspring as early as the 8th week of pregnancy.

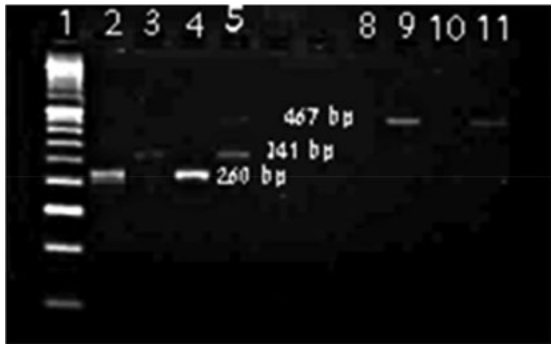

Figure 1. Agarose gel electrophoresis of the PCR product of AMLX/AMLY and TSPY gene from the normal bulls and two sub-fertile cows. Lane1: DNA ladder. Normal bull: Lanes 2 and 4 (TSPY) showed 260 bp, Lanes 3 and 5 (AMLX/AMLY) showed 467/341 bp. Sub-fertile cows: Lanes 8 and 10 (TSPY) showed negative. Lanes 9 and 11 (AMLX): 467bp

The absence of Y chromosome in this study indicated that the sub-fertile cows are free from Y chromosome anomaly. McDaneld *et al.* (2011) reported that there are two possibilities for the presence of Y chromosome gene in some females. The first possibility was at least some of the females were Freemartins. The other possibility was that the fragment of the Y chromosome may have crossed over to the father's X chromosome, which the female

offspring then inherits. This crossover would result in the female offspring having fragment of the Y chromosome in their genome.

Screening of the presence of Y chromosome is an important tool in the determination of sex as well as diagnosing for Y chromosome related genetic abnormalities in an animal in breeding programmes which can lead to better heifer replacement decisions, both for beef and dairy herds. The PCR enables a more simple and rapid method to be used. In this study, amelogenin and testis specific protein Y-encoded gene used as potential gene markers for the Y chromosome to sex various animals, instead of zinc-finger-protein gene (Aasen and Medrano, 1990; Shahrum *et al.,* 1995).

The limitation of this tool is that it can only be used for screening purposes for animal sexing or diagnosing sex chromosome anomalies involving Y chromosome. The result does not reflect other sex chromosome abnormalities such as Turner Syndrome, Trisomy or Klinefelter Syndrome. Since the sub-fertile cows showed negative result, further studies need to be conducted to find other related conditions with the translocations or numerical error in chromosomes that affects bovine fertility, possibly by using more advanced PCR techniques.

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