J. Vet. Malaysia (1995) 7(1):7-9

DETECTION OF CHROMOSOME Y-RELATED GENETIC DEFECT IN AN INFERTILE COW USING POLYMERASE CHAIN REACTION

S. Shaharum[1], O. Abas-Mazni[2], A. Sipat[3], Y.S. Chee[4] and H. Hawari[4]

[1]*Institut Bioteknologi Haiwan Kebangsaan, 27000 Jerantut, Pahang, Malaysia.*
[2]*Biotechnology Center, MARDI, G.P.O. Box 12301, 50774 Kuala Lumpur, Malaysia.*
[3]*Dept. of Biochemistry and Microbiology, Universiti Pertanian Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[4]*Department of Veterinary Services, Exchange Square, off Jalan Semantan, 50630 Kuala Lumpur, Malaysia.*

SUMMARY

The zinc-finger-protein gene was used as a marker for the Y chromosome in a normal male, a normal female and an infertile female cattle. The gene was amplified using polymerase chain reaction (PCR). The PCR product (Zfy) from the genomic DNA of a normal male was a single DNA band of size approximately 440 bp and was cleaved by the restriction enzyme Pst I. The PCR product from the genomic DNA of a normal female was also a single DNA band of size approximately 440 bp but this was not cleaved by Pst I. The PCR product from the genomic DNA of the infertile female was found to be a single DNA band of size approximately 440 bp and was cleaved by Pst I, thus indicating the presence of the Y chromosome. This may account for the infertility of the animal. The approach described here for the diagnosis of Y chromosome abnormality in an infertile cow is rapid and more precise compared to the conventional cytological techniques.

Keywords: Infertility in cows, sex-chromosome anomalies, Y chromosome, zinc-finger protein gene, polymerase chain reaction.

INTRODUCTION

The Y chromosome determines the male sex in mammals, hence males have XY genotype while females have XX genotype. In cattle however, sex chromosome anomalies do occur in an intensive breeding programme. The most commonly reported case of sex chromosome anomaly in cattle is the Freemartin Syndrome (Halnan, 1989) which manifest itself as chimerism of sex chromosomes (XX/XY) between cells.

Other possibilities of sex chromosome anomalies in cattle involving the Y chromosome is the XX/XXY trisomy (Scott and Gregory, 1982) and mosaicism (Dain and Bridge, 1978). The additional Y chromosome, or a fragment of it, frequently results in infertility of the females.

The occurrence of a Y chromosomal abnormality in an infertile cow can be detected by conventional karyotyping analysis. Although accurate, this method is time consuming and require a trained cytogeneticist. Another approach is the use of specific Y chromosome markers to detect the presence of Y chromosome in such an animal. One such marker is the zinc-finger protein gene which has previously been used to determine the sex of various animals, including cattle (Aasen and Medrano, 1990). The zinc-finger-protein gene (Zfy) found in the Y chromosome can be amplified using polymerase chain reaction (PCR) giving a product of 447 bp (Aasen and Medrano, 1990). A homologous gene has also been discovered (Schneider *et al.*, 1989) associated with the X chromosome (Zfx, 445 bp) but this does not have a site for the restriction enzyme Pst I (Aasen and Medrano, 1990). PCR amplification of this gene followed by a Pst I digestion of the product can thus be used to analyse for the presence of the Y chromosome in a DNA sample from an individual animal and hence to determine its sex.

We have pursued this approach further to determine the presence of the Y chromosome in an infertile cow. This animal, which did not conceive after an intensive artificial insemination programme in the farm, was suspected to have chromosome Y-related genetic abnormality. The results indicated that this method is faster and precise compared to conventional karyotyping to diagnose for Y chromosomal abnormality in an infertile crossbred cow.

MATERIAL AND METHODS

Peripheral blood samples were collected in Venoject tubes (heparinised) from a normal male, a normal female and an infertile female animal (I/D. No: 2337, Breed: MAFRIWAL). The blood samples were kept frozen (-20°C) until use. Total DNA was

microfuge tube. 500 µL of total blood sample was mixed with the homogenisation buffer (0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA and 0.3 M Tris-Cl pH 8.0). 500 µL of 10% (w/v) sodium dodecyl sulphate was added to the suspension and the mixture was gently vortexed. The mixture was then incubated at 65°C for 90 min. 200 µL of 5 M ammonium acetate was added and mixed by gentle vortexing. The sample was then cooled on ice for one hour and then centrifuged at 5,000 rpm, 4°C for 10 min. The supernatant was transferred to a new, sterile microfuge tube and extracted twice with phenol and chloroform. The aqueous phase was transferred to a new tube and the DNA precipitated using ice-cold absolute ethanol. The DNA pellet was washed once with 80% chilled ethanol, dried and finally resuspended in 50 µL TE buffer (10 mM Tris-Cl pH 8.0, 1.0 mM EDTA).

Oligonucleotide PCR primers were obtained from Bio-Synthesis, Inc. (Lewisville, TX. USA). The 25 bp primer were designed according to the sequences (Aasen and Medrano, 1990) :

P1-5EZ {5'-ATAATCACATGGAGAGGCACAAGCT-3'}
and
P2-3EZ {5'-GCACTTCTTTGGTATCTGAGAAAGT-3'}

PCR amplification of the Zfy/Zfx gene was carried out using a ThermoJet Thermal Cycler (Equibio, Belgium). The PCR mixture contained 2 µL of DNA [approx. 0.5 µg], 83 µL sterile ddH_2O, 10 µL of PCR buffer (Boehringer Mannheim, Germany), 1 µL of Taq DNA polymerase (5 Unit/µL, Boehringer Mannheim, Germany), 10 µL of 10 mM dNTP mix (dATP, dGTP, dCTP, dTTP), 2 µL of each primer (10 pmole). The PCR reaction was done for 33 cycles at the following cycle profile : denaturation 94°C, 45 sec, annealing 60°C, 45 sec, and extension 73°C, 1 min.

Ten µL of the PCR product was mixed with 5 µL of loading buffer [0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanole, 10% (v/v) glycerol in dH_2O] and then loaded on the 2% (w/v) agarose gel. Electrophoresis was carried out in 1X TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA) at 100 Volts for 40 min. The gel was then stained with ethidium bromide (0.5 µg/mL). The gel was viewed, and photographed over the UV light (312 nm).

Fifteen µL of the PCR reaction mixture was digested at 37°C for at least 3 hours by using 10 Units of Pst I restriction endonuclease (Boehringer Mannheim, Germany) and analysed by 2% (w/v) agarose gel electrophoresis followed by ethidium bromide staining. The DNA bands were photographed over UV light.

RESULTS AND DISCUSSION

The result of a PCR amplification of the zinc-finger-protein gene from a normal female and a normal male cattle using the primers P1-5EZ and P2-3EZ (Aasen and Medrano, 1990) is shown in Figure 1. The size of the Zfx and Zfy gene product obtained was estimated to be about 440 bp (Figure 1, lane 1 and lane 3 respectively). It was previously reported that in cattle and various other animals, the Zfy gene, but not the Zfx gene, has a single Pst I site (Aasen and Medrano, 1990). As shown in Figure 1 (lane 4), cleavage of the PCR product from the male cattle (Zfy) with Pst I produced bands of sizes approximately 440 bp, 340 bp and 100 bp. The latter two fragments were presumably generated from cleavage of the Zfy gene while the 440 bp fragment is from the Zfx gene associated with the X chromosome in the normal male animal (genotype XY). The Zfx gene from the normal female animal was not cleaved by Pst I (Figure 1, lane 2). These results are consistent with those of Aasen and Medrano (1990).

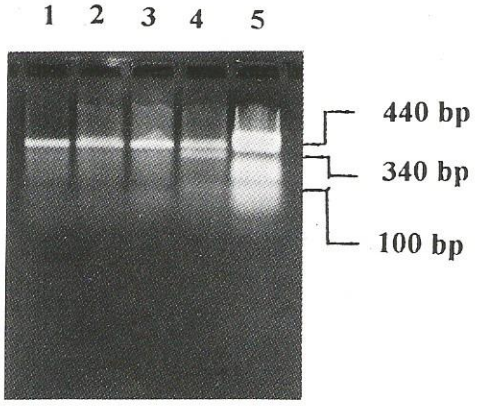

Figure 1. Agarose gel electrophoresis of the PCR product of the Zfx/Zfy gene from the genomic DNA of normal animals.
Lane 1 : uncut PCR product from the female
Lane 2 : Pst I digestion of the PCR product from the female
Lane 3 : uncut PCR product from the male
Lane 4 : Pst I digestion of the PCR product from the female
Lane 5 : DNA size marker (pBR 322 cut with Hae III)

Amplification of the zinc-finger-protein gene from the DNA of an infertile cow suspected of having a Y chromosome abnormality was also carried out. The results in Figure 2, lane 6, show a PCR product of approximately 440 bp, similar to those from the normal female and male (lane 2 and lane 4, respectively). Pst I digestion of the PCR product from the infertile female produced bands of sizes approximately 440 bp, 340 bp and 100 bp (Figure 2, lane 7). This result is similar to that obtained for the normal male animal (Figure 2, lane 5). The largest DNA band (approximately 440 bp, Figure 2 lane 7) had a fluorescence intensity about twice of a normal male. This may correspond to the Zfx gene product that does not cut with Pst I. The high intensity of this band is probably due to the presence of more X chromosomes (either XX/XY or XXY) than the Y chromosome in the genotype of this animal, as compared to normal male which has only the XY genotype.

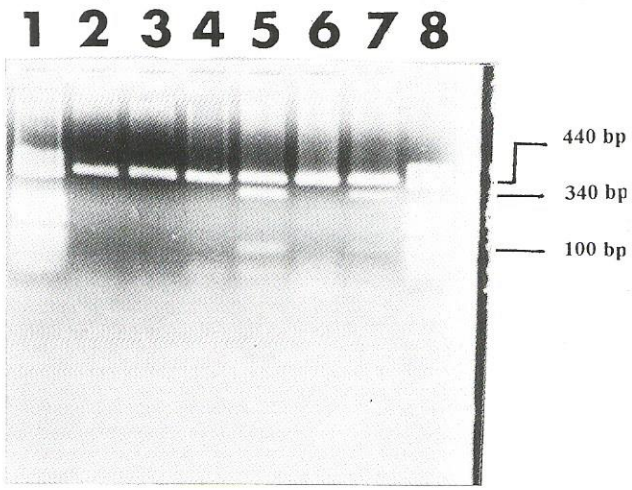

Figure 2. Agarose gel electrophoresis of the PCR product of the Zfx/Zfy gene from the genomic DNA of normal and infertile animals.

Lane 1 and 8: DNA size marker (pBR 322 cut with Hae III)
Lane 2: uncut PCR product from the female
Lane 3: Pst I digestion of the PCR product from the female
Lane 4: uncut PCR product from the male
Lane 5: Pst I digestion of the PCR product from the female
Lane 6: uncut PCR product from the infertile female
Lane 7: Pst I digestion of the PCR product from the infertile female

Screening for the occurrence of the Y chromosome is an important procedure in the determination of sex as well as in diagnosing for Y chromosome related genetic abnormalities in an animal breeding programme. This can be done by a cytological examination of tissue samples. The advent of the PCR technique however, enables a more simple and rapid method to be developed. Aasen and Medrano (1990), therefore, used the zinc-finger-protein gene as a marker for the Y chromosome to sex various animals since the male gene Zfy can be distinguished from female allele Zfx by the use of the restriction enzyme Pst I.

This approach has been further pursued in the present work to establish the presence of the Y chromosome in an infertile female cow. Using PCR and the primers for the Zfy/Zfx genes, it was shown that the animal has a Y chromosome, which may have accounted for the infertility. This technique can be used as a rapid and precise method for screening of `chromosome Y-related' genetic defect among crossbred cows by amplification of Zfy/Zfx genes.

ACKNOWLEDGEMENTS

This work was funded by the Department of Veterinary Services, Malaysia. Sincere appreciation is expressed to Dr. Khatijah Mohd. Yusuf and Dr. Norani Abdul Samad, Department of Biochemistry and Microbiology, Universiti Pertanian Malaysia for the use of their laboratory facilities.

REFERENCES

Aasen, E. and Medrano, J.F. (1990). Amplification of Zfy and Zfx genes for sex determination in human, cattle, sheep and goat. *Biotechnology*, **8**: 1279-1281.

Dain, A.R. and Bridge, P. S. (1978). A chimeric calf with XX/XXY mosaicism and intersexuality. *J. of Reproduct. Fert.*, **54**: 197-201.

Halnan, C.R.E. (1989). Karyotype and phenotype in cattle and hybrid of the genus. *In*: Animal Cytogenetics. CAB International. pp 237-240.

Schneider, G.A., Romero, B.P., Brown, L.G., Nussbaum, R. and Page, D.C. (1989). Zfx has a gene structure similar to Zfy, the putative sex determinant and escape X inactivation. *Cell*, **57**: 1247-1258.

Scott, C.D. and Gregory, P.W. (1982). An XXY trisomic in an intersex of *Bos taurus*. *Genetics*, **52**: 473-474.

RINGKASAN

PENGESANAN KECACATAN GENETIK TERKAIT KROMOSOM Y DALAM LEMBU TAK SUBUR MENGGUNA TINDAK BALAS RANGKAIAN POLIMERASE

Gen `zinc-finger-protein' telah diguna sebagai penanda kepada kromosom Y dalam seekor lembu jantan normal, seekor lembu betina normal dan seekor lembu betina tak subur. Gen ini telh diperbanyakkan melalui tindak balas rangkaian polimerase (PCR). Hasil PCR (Zfy) daripada DNA genom lembu jantan normal merupakan satu jalur DNA tunggal bersaiz lebih kurang 440 bp dan dapat dibelah oleh enzim pembatas Pst I. Hasil PCR dari DNA genom lembu betina normal juga merupakan satu jalur DNA bersaiz lebih kurang 440, bp tetapi ianya tidak dibelah oleh enzim pembatas Pst I. Hasil PCR daripada DNA genom lembu betina tak subur didapati berbentuk jalur DNA tungggal bersaiz 440 bp yang tidak dibelah oleh Pst I dan ini menunjukkan wujudnya kromosom Y. Penemuan ini mungkin menerangkan ketaksuburan haiwan tersebut. Pendekatan yang dihuraikan untuk diagnosis keabnormalan kromoson Y dalam lembu tak subur ini adalah cepat dan lebih tepat berbanding teknik sitologi biasa.