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Single nucleotide polymorphism (SNP) analysis of ovine progesterone receptor gene in Malpura ewes

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Progesterone hormone, critical for maintenance of pregnancy (Wetendorf and Demayo 2012), acts by induction of complex paracrine signalling pathway mediated by its cognate progesterone receptor (PGR). PGR plays crucial role in establishment of conceptus inside uterus (Brooks *et al*. 2014). In the present study, nucleotide sequences of ligand binding domain (partial) of ovine PGR gene from repeat breeder Malpura ewes were analyzed for single nucleotide polymorphism (SNP) and its association with repeat breeding.

Malpura ewes were selected for study based on clinical history of repeat breeding. Blood samples from true cyclic and repeat breeders were collected from the institute farms and DNA was extracted using standard phenol-chloroform method (Sambrook and Russell 2001). Quality and quantity of DNA were ascertained using agarose gel electrophoresis and spectrophotometric analysis, respectively. Primers were designed from ovine PR sequences available in GenBank using primer3 (http://bioinfo.ut.ee/primer3/) tool. Primer encompassing exon 3 viz: forward 5'- atcaggtggccaaggtattg - 3' and reverse 5'- tcattattttcaatactgccaatgt - 3' (product size 627 bp) were used for amplification of ovine PGR gene from fertile and repeat breeder ewes. PCR reaction was performed in gradient thermal cycler programmed for initial denaturation at 94°C for 7 min followed by 35 cycles of 94°C for 45 sec, 57°C for 40 sec and 72°C for 40 sec with a final extension at 72°C for 5 min. High fidelity DNA polymerases were used for amplification of gene sequences. PCR products were resolved on 1.5% agarose gel and excised out from gel on UV illuminator. PCR products were gel extracted and purified using gel extraction kit and were sequenced. Nucleotide sequences were aligned with ovine progesterone receptor gene sequences available in GenBank using BLAST programme (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignment and phylogenetic study was conducted using clustal Omega multiple sequence alignment (http://www.ebi.ac.uk/) tool. Single nucleotide polymorphism was noted manually and location of domain of the deduced amino acids was confirmed from NCBI's conserved domain database (http: //www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml).

PGR is ligand activated transcription regulator and play a key role in crucial physiological functions related to growth and reproduction. It plays key role in the initiation and maintenance of pregnancy and in embryo development (Brooks *et al*. 2014). The activated progesterone receptor is the master regulator of the endometrium waves of intense tissue remodelling signalling onset of pregnancy which is highly regulated activity controlled by cellular molecules (Al-Sabbagh *et al*. 2012). The ability of PGR to activate transcription of target genes requires the binding of cognate ligands (progesterone) to their ligand binding domain (LBD). Progesterone binds with LBD of PR which alters transcription of a variety of genes in target tissues through downstream signalling involving multiple signalling pathways. Any alteration in LBD cause due to single amino acid change can affect binding ability of the hormone with its cognate receptors. SNPs in the intron 3 and 4 of PGR gene were correlated with fertility traits in dairy cattle (Driver *et al*. 2009, Yang *et al*. 2011). In the present work, PCR amplification of progesterone receptor gene (627 bp fragments) was done (Fig. 1) from selected Malpura ewes (40) with known clinical history of cyclic (20) and repeat breeding (20). A total of 40 sequences of Ovine PGR gene were aligned and SNPs (13) were noted, mostly in intronic region. Different allelic sequences were submitted to the NCBI with accession numbers KU946965, KU946966, KU946967, KU946968, KU946969 and KU946970. Two novel missense substitutions were noted in the exon 3 where histidine (H) was replaced with tyrosine (Y). Additionally, these nucleotide variations were associated with clinical history of repeat breeding of the ewes; however, no association was established with any of the 13 SNPs identified. Furthermore, phylogenetic analysis of different alleles of Malpura ewes was compared with PGR amino acids sequences of different domestic animals (Fig. 2).

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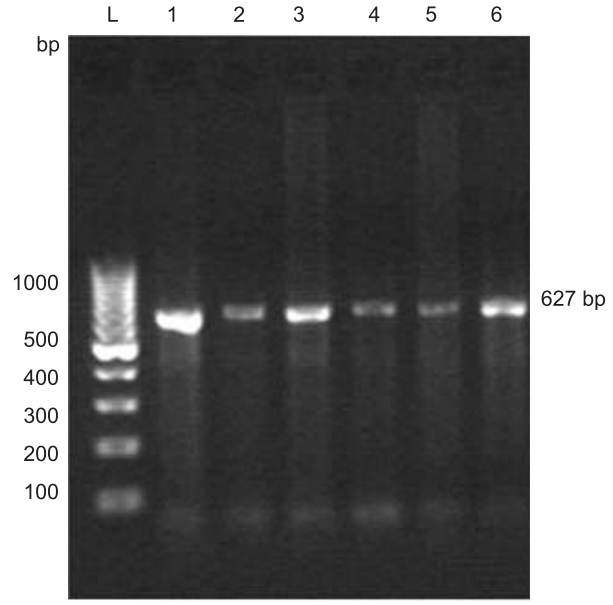

Fig. 1. PCR products of progesterone receptor gene from Malpura sheep resolved on 2% agarose gel electrophoresis. Lane 1-100 bp DNA ladder; lane 2, 6 PCR products of progesterone receptor gene amplified from blood genomic DNA of Malpura ewes.

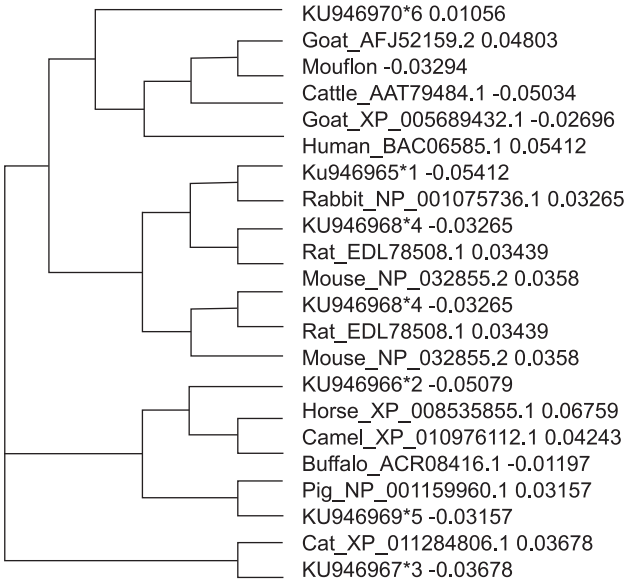

Fig. 2. Phylogenetic analysis of progesterone receptor alleles (KU946965*1, KU946966*2, KU946967*3, KU946968*4, KU946969*5 and KU946970*6) of Malpura sheep with corresponding progesterone receptor sequences available in NCBI.

Allele KU946970*6 did not correspond to any of the studied species and was placed in a separate cluster. This may be a unique variation in Malpura sheep with amount of genetic change not observed in others. A complete PGR gene sequence analysis from infertile ewes can be of great help to find putative role of PGR gene sequence variation in true cyclic and repeat breeder ewes.

SUMMARY

In the present work, nucleotide sequences of progesterone receptor gene (627 bp fragments) encompassing partial ligand binding domain from repeat breeder Malpura ewes were analyzed for presence of SNPs and its association with repeat breeding. A total of 40 sequences were aligned and 13 SNPs were noted mostly in intronic region. Two novel missense mutations were observed in the ligand binding domain where histidine (H) was replaced with tyrosine (Y). These SNPs were not associated with repeat breeding in Malpura ewes.

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