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PGR polymorphism in exon 3 correlated with reproductive traits in Murrah buffaloes

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Abstract

Progesterone receptor (PGR) has been known to play a central role in reproduction, being involved in ovulation, implantation, and pregnancy. Progesterone is essential for the development of decidual tissues, and if fertilization occurs, high circulating progesterone levels are important not only for facilitating implantation, but also for maintaining pregnancy. The present study was aimed to identify and characterize the PGR gene exon 3 by Bsp HI and Hpa II restriction enzymes based on PCR-RFLP. Sixty five Murrah buffaloes were selected and divided in to six groups on the basis of their physiological data. We found high genetic variation ranging from 0% to 1.7% (nucleotide sequence) and 0% to 8.1% (protein sequence) for exon 3 region of PGR gene. A nonsynonymous SNP was observed in exon 3 at 106 (A→G). Entropy plots also confirmed the genetic variation via peaks and the area covered by those peaks.

Keywords: *Buffalo, PGR, PCR-RFLP, SNP detection.*

Introduction

A larger part of the human population depends on domestic water buffalo than on any other livestock species in the world. This species was distributed from southern Asia to Europe during the Pleistocene. But later on, its distribution was restricted to the Indian subcontinent and Southeast Asia with the increase of dry climatic conditions (Kumar *et al*., 2006). The water buffalo (*Bubalus bubalis*) contributes immensely to the agricultural economy through milk, meat, hides and draught power. River buffalo (2N= 50), a subtype of water buffalo have high lactation yields and are more suited to ploughing and drafting on dry plane land, along with for milk and meat purposes (Amaral *et al*., 2007). India possesses the best river buffalo breeds (Murrah) in Asia, which originated from the north-western states of India and have a high potential for milk and fat production apart from their use as a work animal and as a supplementary stock for use as meat production (Sethi, 2003; Rosati and Van Vleck, 2002). Despite having more than fifty percent of milk production alone among total cattle population (Mitra *et al*., 2012), efficiency of milk production remains low due to absence of appropriate selection of genetically superior animals.

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Reproductive efficiency is the primary factor influencing productivity and is hampered in female buffalo by delayed maturation, silent estrus, low conception rates and prolonged inter calving intervals (Oswin Perera, 1999). Improvement of reproductive efficiency in female buffaloes requires a better understanding of their reproductive physiology under steroid hormonal control, especially in the oviduct, during oestrous cycle.

Progesterone plays a central role in reproductive biology of vertebrates, including mammals and is found associated with pregnancy establishment and maintenance (Conneely, 2002) along with the complex regulation of the reproductive events including release of mature oocytes, facilitation of implantation, and maintenance of pregnancy, by promotion of uterine growth and suppression of myometrial contractility (Graham and Clarke, 1997). Its functioning is mediated by specific receptors that are localized in or near the nucleus of target cells and form a super family of nuclear transcription factors (Katsu *et al*., 2010). Progesterone receptor (PGR) gene was hence considered to be a candidate gene (Gutierrez-Sagal *et al*., 1993). Receptors for progesterone are expressed as two distinct isoforms, PR-A and PR-B that arise from a single gene (Conneely *et al*., 1989; Kastner *et al*., 1990). However, the ratios of the individual isoforms vary in reproductive tissues as a consequence of developmental (Shyamala *et al*., 1990)

and hormonal status (Duffy *et al*., 1997) and during carcinogenesis (Brandon *et al*., 1993; Graham *et al*., 1996; Vang *et al*., 2023). The PGR gene has been studied for polymorphism in chicken (Conneely *et al*., 1989; Huckaby *et al*., 1987; Dominguez-Steglich *et al*., 1992), mouse (Naylor *et al*., 1989; Schott *et al*., 1991; Lydon *et al*., 1995), rabbit (Misrahi *et al*.,1987; Peiro *et al*., 2008; Loosfelt *et al*., 1986), human (Misrahi *et al*., 1987; Rousseau-Merck *et al*., 1987; Langmia *et al*., 2015; Asmarinah *et al*., 2017) and crocodile (Suwattana *et al*., 1999).

In order to enhance genetic merit of animals with increased milk production, enhanced reproductive efficiency, disease resistance etc, it is important to identify and locate responsible gene quantitative trait loci (QTL) in the genome. Detection and utilization of available genetic polymorphisms using molecular genetic techniques may play an important role as genetic markers in many fields of animal breeding. A number of techniques were adopted to detect polymorphism at structural loci, of which polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) is the most preferred method (because of its simplicity, quickness, economical, very high repeatability and non-use of hazardous radioactive material) for SNP genotyping and mutation detection. The discovery of RFLP generated renewed interest in the use of gene marker loci as an aid to selection programmes. In view of this, the present study was designed to detect the presence of any genetic variation in PGR gene exon 3 in association with reproductive traits.

Materials and Methods

Germplasm collection

A total numbers of 65 Murrah buffaloes were taken in the present study. Out of which 14 heifers and 21 multiparous buffaloes were selected from the CIRB (Central Institute for Research on Buffaloes) herd. Thirty multiparous buffaloes were selected from different regions of Haryana state and were reared on standard farm management conditions at CIRB, Hisar. Data obtained related to animals details was subjected to analysis of variance (ANOVA) and mean ±SE were ranked using Duncan's multiple range test according to SPSS 16.0 (Table 1). All these animals were further divided into six groups on the basis of their conception and calving after parturition as follows: Group: I with 8 animals (Multiparous normal buffaloes; MN), Group: II with 13 animals (Multiparous abnormal buffaloes; MA), Group: III with 10 animals (Multiparous normal buffaloes from the field; FN), Group: IV with 20 animals (Multiparous abnormal buffaloes from the field; FA) , Group: V with 7animals (Heifer normal; HN), Group: VI with 7 animals (Heifer abnormal; HA).

DNA extraction and Primers designing

DNA was extracted from Fresh blood samples by method of Sambrook and Russel (Sambrook and Russel, 2001) with slight modifications. The quality and quantity of extracted DNA was checked by Nano drop spectrophotometer and agarose gel electrophoresis. The genomic region of PGR gene exon 3 was amplified by PCR with primers designed using Primer 3 software (Table 2) from the *Bos Taurus* gene sequence (Accession no: NM_001205356.1, Chromosome 14) (Fig 1). Due to the greater percentage similarity of conserved DNA sequences (75%) in Bovidae family which includes cattle, bovine and buffalo, the bovine was considered as a reference animal (Navani *et al*., 2002). The region was focused because of its presence on ligand binding site.

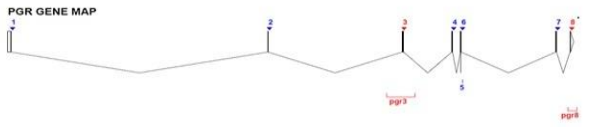

Fig 1 PGR gene map of Bovine

PCR amplification

The PCR was carried out for PGR exon 3 in a final volume of 15.0 µl consisted of 80 ng genomic DNA, 1.25 pm each primer, 2.0 mM $MgCl_2$, 0.15 mM dNTPs, 1X PCR buffer and 1 U Taq polymerase. The thermal profile consisted of initial denaturation (94˚C for 5 minutes) followed by 35 cycles of denaturation (94˚C for 45 seconds), annealing (62.7^0C for 50 seconds), extension (72˚C for 50 seconds) and final extension (72˚C for 7 minutes). The PCR amplification was confirmed by restriction digestion of PGR exon 3 amplicons with *Bsp* HI enzyme. The final volume of digestion reaction was 15 µl, containing 5 µl reaction solution, 1 µl enzyme buffer, 1 µl enzyme and 8 µl nuclease free water and incubated at 37˚C for approximately 3 hours. The reaction was stopped by addition of 4 µl of 6x gel loading dye and freezing the content at -20˚C. After digestion, the samples were quantified to visualize the amplified fragments by gel electrophoresis with the 100bp ladder DNA marker.

Restriction Fragment Length Polymorphism (RFLP)

The amplicons were screened for the presence of genetic polymorphism in PGR via PCR-RFLP. The progesterone receptor gene PCR product (exon 3) was digested with *Hpa* II (5'C↓CGG3') for genotyping in a final reaction volume of 25 µl, containing 15 µl reaction solution, 2.5 µl enzyme buffer, 0.8 µl enzyme and 6.7 µl nuclease free water and incubated at 37˚C for overnight. The cleaved fragment were separated by electrophoresis on 2% agarose gel in 1X TAE buffer containing 0.05µg/mL ethidium bromide at 90V for approximately 1 hour. The bands were visualized under ultraviolet light in Gel-Doc system (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

The PCR product was purified by QIAquick kit (Qiagen). The purified amplified PCR products of PGR gene were

sequenced from Xcelris Labs Ltd., Ahmedabad. Both the DNA strands (i.e. forward and reverse) were sequenced and were assembled using Codon Code Aligner 5.0.1. The assembled sequence were then compared among themselves and also with the bovine reference followed by detection of mutations (SNPs) via Codon Code Aligner 5.0.1.

Table 1 Parameters of animals details (Mean±SE)

Group	Lactation No.	Calving Interval **	Lactation Length *	Total Lactation Yield	305 Days lactation yield	Peak Milk Yield	Dry Period	Open Days
Multiparous normal (n=8)	3.75±0.65^{a}	409.26±12.47^{a}	303.15±8.26^{a}	1764.50±166.27^{a}	1694.00±157.21	9.15±0.63^{a}	121.75±16.58^{a}	149.57±27.10^{a}
Multiparous abnormal (n=13)	3.15±0.37^{ab}	603.17±26.80^{a}	385.18±23.24^{b}	2555.30±164.96^{b}	2166.40±111.17	9.90±0.53^{ab}	230.26±29.53^{a}	314.02±24.53^{b}
Field - Normal(n=10)	3.90±0.53^{a}	392.51±15.60^{a}	270.33±13.64^{a}	2271.20±230.65^{ab}	2206.40±215.40	11.91±0.89^{c}	158.31±27.62^{a}	268.16±66.80^{ab}
Field – Abnormal (n=20)	2.25±0.36^{b}	915.08±121.0^{b}	277.97±25.25^{a}	2158.00±221.66^{ab}	2018.40±204.11	11.61±0.45^{bc}	391.74±78.22^{b}	255.15±57.68^{ab}

** Significant (p<0.01) difference between groups
* Significant (p<0.05) difference between groups
a,b,c superscripts differed significant (p<0.05)
The age of the heifers was 44.06±2.71 months and multiparous buffaloes were 47.67±1.13 months.

Table 2 Primer sequence and thermal profile for the amplification of PGR gene

Primer	Sequences 5′ -------- 3′	Gene	Region/Locus	PCR conditions	PCR product size
PP3f PP3r	5′-TAATCCATGTCTGAGACCTAACC-3′ 5′-ATTTAGCCACCCACTAGTATGAAG-3′	PGR Exon-3	Partial intron 2-3, exon 3, partial intron 3-4	94°C 45 sec 62.7°C 50sec 72°C 50 sec	560bp

Further, these sequences were aligned and compared using Bio edit software and multiple alignment programs, Clustal W (http://www.ebi.ac.uk/Tools/msa /clastalw2/) with DNA weight matrix and multiple parameters like gap opening 10.0, gap extension 0.20, and transition weight 0.50. Pairwise sequence identity, divergence of PGR gene among different groups of buffalo and Entropy plot of PGR gene was calculated using BioEdit software and compared with the reference sequence.

A phylogenetic tree among the different groups of buffalo was constructed based on pairwise similarity coefficients by neighbour-joining/ UPGMA method.

Results

A total of 65 animals were used in the present study which were divided into six groups.

Table 3 Values of most favoured region, generously allowed regions and disallowed regions and Errat score (% age)

Sample	Most favoured regions	Generously allowed regions	Disallowed regions	Errat score
MN	74	3.3	0.4	**47.131**
FN	74.4	2.9	0.8	**64.634**
HN	73.6	2.5	0.4	**67.480**
MA	73.1	3.3	0.4	**48.770**
FA	70.5	2.1	0.4	**49.593**
HA	74.7	2.5	0.4	**51.600**

The mean conception age of normal buffalo heifers was significantly (p<0.01) lower (35.90±1.46) than the abnormal heifers (54.25±3.16). Calving interval also differed significantly (p<0.01) between groups and within the groups. The lactation length also differed significantly (p<0.05) between defferent groups. Mean calving interval, lactation length, total lactation yield, peak milk yield, dry period and open days were calculated (Table 1). The animals were genotyped for the presence of any genetic varaiation and its association with reproductive traits. PGR gene exon 3 from different buffalo groups was partially amplified using specifically designed primers for concerned PGR region (Table 3).

The PCR amplification of PGR exon 3 produced a clear, distinct and highly reproducible band of 560 bp. The amplified products were confirmed by two-way nucleotide sequencing using the same set of primers and by comparing with the reference gene sequence of bovine. PCR-RFLP of the amplicon was carried out using *Hpa* II (5′C$^{\downarrow}$CGG3′) and produced two fragments of 249 bp and 311 bp in all samples except field abnormal in which three fragments were produced i.e 276 bp, 249 bp and 35 bp (Fig 2).

Fig 2 PCR-RFLP pattern of PGR exon 3 gene. A representative gel pictures showing the *Hpa* II restriction digested fragment of PGR exon 3 from different groups of Murrah buffalo: 1-2 shows HN, 3-4 shows HA, 5-6 FN, 7-8 MN and MA groups, 9-12 shows FA ,13 shows uncut amplicon. M: 100bp ladder.

This indicated that the digestion was not in accordance with the data from web cutter analysis. However, these restriction digestion patterns exhibited monomorphic pattern in all animals under study. But when the contigs of PGR sequence of different groups of Murrah buffalo was then compared with that of bovine (Acc: NM_001205356) sequence to annotate different exonic regions putatively to identify SNPs in respective region. A very few variations were observed among PGR sequences of different animal groups. All the nucleotide sequences of complete amplicon (560 bp) were compared via multiple sequence alignment programs using BioEdit 5.0.1 version and level of variations in nucleotide sequences were detected (Fig 3).

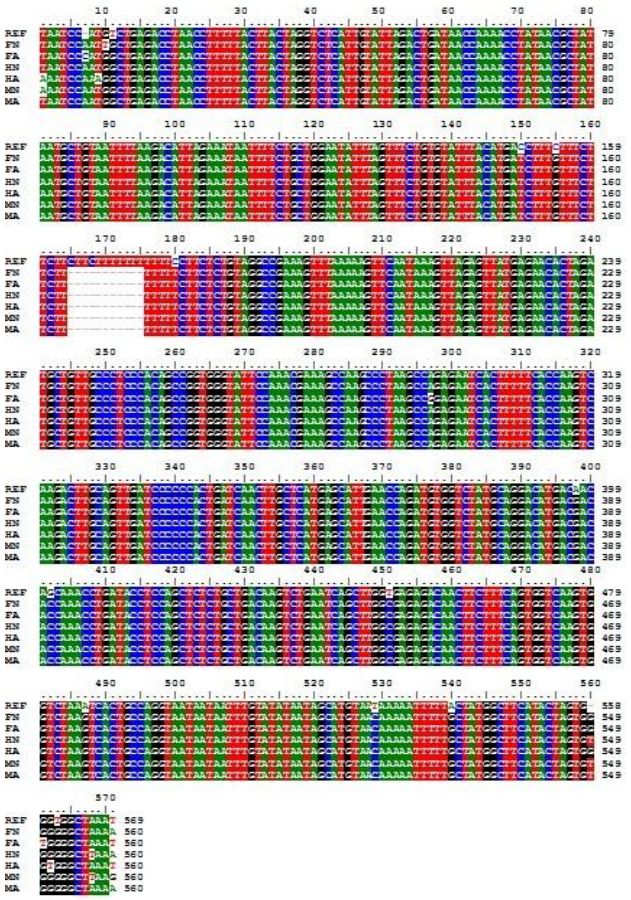

Fig 3 Multiple sequence alignment of PGR-exon 3 (560 bp) complete amplicon obtained from different groups of buffaloes with reference. REF: Bovine, MN, MA, HN, FN, FA, HA

Furthermore, the exonic nucleotide sequence of PGR gene was also compared (Fig 4) and then the exonic DNA was then conceptually translated and compared with that of the bovine to detect amino acid changes in buffalo PGR region under study (Fig 5).

The pair wise genetic variation on the basis of PGR sequence (exon-3) was calculated among different groups and was found to vary from 0.0% to 1.7%. FN, HN, MA, HA and MN groups showed lowest value of distance matrix (0.0%) indicating more similarity among themselves, whereas FA group showed more dissimilarity from all of these groups (0.3%). However, reference showed different value of distance matrix for FN, HN, MA, HA and MN groups (1.3%) and for FA group (1.7%) (Fig 6).

The dendrogram generated on the basis of DNA sequence similarity showed that MN, HA, MA and HN groups were very close to each other and to that of reference along with FN group. Whereas, FA group was distantly placed from reference (Fig 6).

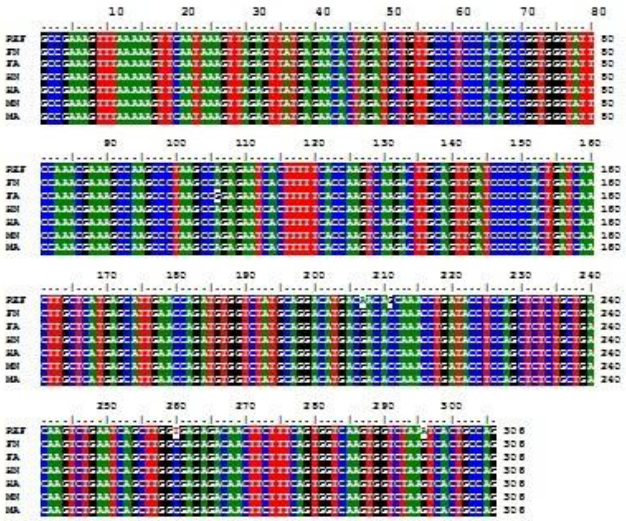

Fig 4 Multiple sequence alignment of PGR exon-3 obtained from different groups of buffaloes with reference. REF: Bovine, MN, MA, HN, FN, FA, HA

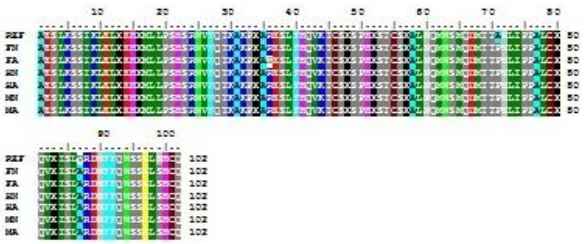

Fig 5 Multiple sequence alignment of PGR protein sequence from different groups of buffaloes with reference. REF: Bovine, MN, MA, HN, FN, FA, HA

Fig 6 Dendrogram showing similarity between PGR gene exon 3sequences obtained from different groups of buffaloes. REF: Bovine, MN, MA, HN, FN, FA, HA

Based upon the protein sequence comparison, a very small variation was found among different groups under study. Overall variation ranged from 0.0% to 8.1%. FN, HN, MA, HA and MN groups were more similar to each other with lowest value of distance matrix (0.0%), whereas FA group showed more dissimilarity from all of

these groups (1.9%). However, reference showed different value of distance matrix for FN, HN, MA, HA and MN groups (6.0%) and for FA group (8.1%). The dendrogram generated on the basis of protein sequence similarity showed that FN, HA, MN, and HN groups were very much close to each other. They were close to reference along with MA group with variable similarity of protein sequence. FA group was placed far apart from all other groups (Fig 7).

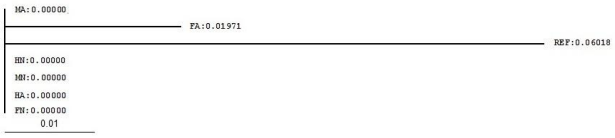

Fig 7 Dendrogram showing similarity between PGR protein sequences obtained from different groups of buffaloes. REF: Bovine, MN, MA, HN, FN, FA, HA

SNP detection

Clustal W multiple alignments with bovine sequence revealed only single SNP at position 106 A→G in FA (Fig 4). When these sequences were translated and compared with bovine the FA showed that the change at position 36 i.e. Glycine (G) instead of Arginine (R). So we observed only single non synonymous mutation. We also observed deletion of 10 bases (165-175 bp) in intron region (Fig 3) among all groups when compared to the reference sequence (bovine).

Entropy plot of PGR exon 3 sequences

The peaks in the entropy plot were present only at that position where any of the sequence under study showed some variation from that of the reference (both in case of DNA and protein) the area of the plot also got increased with the increase in level of variation. The entropy plot showed zero value due to the conserved sequence. The peaks however showed variations in the nucleotide sequence with reference and also among the groups at positions 106, 207, 211, 260 and 296 whereas at position 36, 71, 87 and 99 in case of amino acid sequence (Fig 8 and 9).

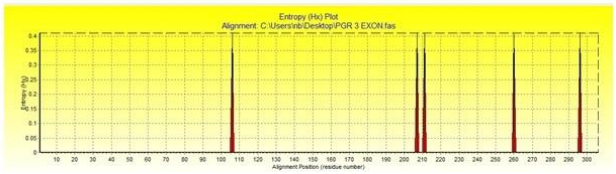

Fig 8 Entropy plot showing variability in PGR exon 3 nucleotide sequence alignments with reference

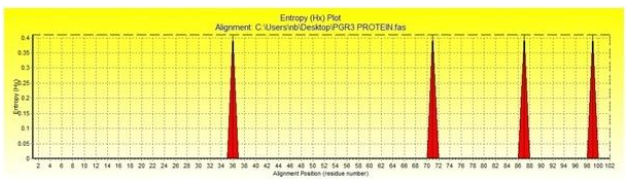

Fig 9 Entropy plot showing variability in PGR exon 3 protein sequence alignments with reference

Percentage identity and divergence of PGR gene exon 3 and proteins among various groups

Percent identity and divergence was computed by BioEdit software among different animal groupings. When the percent nucleotide identity of FN group was calculated with other groups it was found that this group showed maximum identity (100%) with HN, HA, MN and MA groups and lower identity (99.6%) to that of FA group, whereas minimum identity (98.6%) was shown with reference. FA group was found to show maximum identity (99.6%) with HN, HA, MN and MA groups and 98.3 % identity with reference. HN group showed 100% identity with HA, MN and MA groups and 98.6% identity with reference. HA group showed 100% identity MN and MA groups and 98.6% identity with reference. MN group was found to show 100% identity with MA group and 98.6% identity with that of reference. MA group showed 98.6% identity with reference (Fig 10).

Percent Identity

Distance Mattrix

	FN	FA	HN	HA	MN	MA	REF	
FN		99.6	100	100	100	100	98.6	FN
FA	0.3		99.6	99.6	99.6	99.6	98.3	FA
HN	0.0	0.3		100	100	100	98.6	HN
HA	0.0	0.3	0.0		100	100	98.6	HA
MN	0.0	0.3	0.0	0.0		100	98.6	MN
MA	0.0	0.3	0.0	0.0	0.0		98.6	MA
REF	1.3	1.7	1.3	1.3	1.3	1.3		REF
	FN	FA	HN	HA	MN	MA	REF	

Fig 10 Representative of percent identity and distance matrix of DNA of PGR exon 3 among various groups of buffaloes. REF: Bovine, MN, MA, HN, FN, FA, HA

When percent protein identity of FN group was compared with other groups it was found that this group showed 100% identity with HN, HA, MN and MA groups, followed by 98.9% identity with FA group and 96.8% identity with that of reference. FA group showed 98.9% identity with HN, HA, MN and MA groups and 95.7% identity with reference. HN group was found to show 100% identity with HA, MN and MA groups and 96.8% identity with reference. HA group showed 100% identity with MN and MA groups and 96.8% identity with reference. MN group showed 100% identity with MA group whereas 96.8% identity with that of reference. MA group showed 98.6% identity with reference (Fig 11).

Percent Identity

Distance Matrix

	FN	FA	HN	HA	MN	MA	REF	
FN		98.9	100	100	100	100	96.8	FN
FA	1.9		98.9	98.9	98.9	98.9	95.7	FA
HN	0.0	1.9		100	100	100	96.8	HN
HA	0.0	1.9	0.0		100	100	96.8	HA
MN	0.0	1.9	0.0	0.0		100	96.8	MN
MA	0.0	1.9	0.0	0.0	0.0		96.8	MA
REF	6.0	8.1	6.0	6.0	6.0	6.0		REF
	FN	FA	HN	HA	MN	MA	REF	

Fig 11 Representative of percent identity and distance matrix of protein of PGR exon 3 among various groups of buffaloes. REF: Bovine, MN, MA, HN, FN, FA, HA

Discussion

Fertility in water buffalo (*Bubalus bubalis*) is considerably lower than that in cattle (*Bos taurus* and *Bos indicus*). Furthermore, the reproductive performance of animals is found to get affected by many factors such as nutrition, management and environment. Poor breeding efficiency is attributed to late onset of puberty, seasonality and poor oestrus detection which is a prerequisite for efficient reproductive management particularly in determining optimal mating time. In present study all the groups were found to be monomorphic at the restriction site. However, in field abnormal group, one SNP was observed in exon 3 at position 106 A→G resulting into change in Arginine (R) into Glycine (G) at codon 36. The SNP found in the exon 3 of FA group was not observed in MA and HA groups. The present study in accordance with another study showed the role of environmental factors in reproductive parameters (Aziz et al., 2001). The observed SNP was further found related with reduced amount of gene transcript and decreased protein activity (Romano *et al*., 2007). The promoter region and exon 1-8 in rabbit was studied and detected one SNP 2464 G > A in promoter region, 3 SNP in 5' UTR exon 1 and one silence SNP in exon 7, suggesting the role of the SNP in divergently selected lines for uterine capacity. The observed SNP found in the promoter region was directly associated with high line (H) (higher litter size and higher number of embryo implantation) than the low line (L). SNP in promoter region was found in direct association with litter size (Peiro et al., 2008).

Conclusion

The present study contributes significantly in analysing the polymorphism in PGR gene (which proved as a one of the suitable candidate gene for its association with reproductive efficiency), however the polymorphism study on a bigger population may prove more helpful. These molecular biological studies in association with latest technologies like artificial insemination, super-ovulation, ovum pick-up, *in vitro* maturation, *in vitro* fertilization and embryo transfer will certainly enhance and stabilize the reproductive efficiency of buffalo. The exonic region choosen was present at 3' region of the gene which is actually the ligand binding site and contributes significantly in the reproductive performance of the animal. Some of the animals belonging to the field abnormal group in the present study possessed significant irregularities in reproductive traits like long calving intervals, long dry periods and large number of open days, which may be affected by environmental factors. Hence the SNP detected in the field abnormal animals might be association with reproductive traits. India is gifted with rich genetic resources in terms of its buffalo breeds (especially Murrah), an integral part of agriculture in India. Thus, suitable conservation efforts have to be appropriately focused towards their genetic improvement.

References

[1] Amaral, M.E.J., Owens, K.E., Elliott, J.S., Fickey, C., Schaffer, A.A., Agarwala, R., and Womack, J.E. (2007). Construction of a river buffalo (*Bubalus bubalis*) whole-genome radiation hybrid panel and preliminary RH mapping of chromosomes 3 and 10. *Ital J Anim Sci*, 6 (2), 237-245.

[2] Asmarinah, Rahmala, F.R., Aisyah, R., Hadining, P.A. and Raden, M. (2017). Progesterone Receptor Gene Polymorphisms of rs1042838 and New Mutation in Ovarian Endometriosis in Indonesian Women. Advanced Science Letters, 23(7), 6906-6908.

[3] Aziz, M.A., Schoeman, S.J., Jordaan, G.F., El-Chafie, O.M. and Mahdy, A.T. (2001). Genetic and phenotypic variation of some reproductive traits in Egyptian Buffalo. *S Afr J Anim Sci*, 31(3), 195-199.

[4] Brandon, D.D., Bethea, C.L., Strawn, E.Y., Novy, M.J., Burry, K.A., Harrington, M.S., Ericksonm, T.E., Warner, C., Keenan, E.J. and Clinton, G.M. (1993). Progesterone receptor messenger ribonucleic acid and protein are over expressed in human uterine leiomyomas. *Am J Obstet Gynecol*, 169, 78–85.

[5] Conneely, O.M., Kettelberger, D.M., Tsai, M.J., Schrader, W.T. and Omalley, B.W. (1989). The chicken progesterone receptor A and B isoforms are products of an alternate translation initiation event. *J Biol Chem*, 264, 14062–14064.

[6] Conneely, O.M., Mulac-Jericevic, B., DeMayo, F., Lydon, J.P., Omalley, B.W. (2002) Reproductive functions of progesterone receptors. *Recent Prog Horm Res*, 57, 339-355.

[7] Duffy, D.M., Wells, T.R., Haluska, G.J. and Stouffer, R.L. (1997). The ratio of progesterone receptor isoforms changes in the monkey corpus luteum during the luteal phase of the menstrual cycle. *Biol Reprod*, 57, 693–699.

[8] Graham, J.D. and Clarke, C.L. (1997). Physiological action of progesterone in target tissues. *Endocr Rev*, 18 (4), 502-519.

[9] Graham, J.D., Yeatesm, C., Balleine, R.L., Harvey, S.S., Milliken, J.S., Bilous, A.M. and Clarke, C.L. (1996). Progesterone receptor A and B protein expression in human breast cancer. *J Steroid Biochem Mol Biol*, 56, 93–98.

[10] Gutierrez-Sagal, R., Perez-Palacios, G., Langley, E., Pasapera, A.M., Castro, I., and Cerbon, M.A. (1993). Endometrial expression of progesterone receptor and uteroglobin genesduring early pregnancy in the rabbit. *Mol Reprod Dev*, 34, 244-249.

[11] Kastner, P., Krust, A., Turcotte, B., Stropp, U., Tora, L., Gronemeyer, H., and Chambon, P. (1990). Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J*, 9, 1603–1614.

[12] Katsu, Y., Matsubara, K., Kohno, S., Matsuda, Y., Toriba, M., Oka, K., Guillette, L.J., Ohta, Y., and Iguchi, T. (2010). Molecular Cloning, Characterization, and Chromosome Mapping of Reptilian Estrogen Receptors. *Endocrinology*, 151(12), 5710–5720.

[13] Kumar, S., Gupta, J., Kumar, N., Dikshit, K., Navani, N., Jain, P., and Nagarajan, M. (2006). Genetic variation and relationships among eight Indian reverine buffalo breeds. *Mol Biol*, 15, 593-600.

[14] Langmia, I.M., Apalasamy, Y.D., Omar, S.Z. and Mohamed, Z. (2015). Progesterone Receptor (PGR) gene polymorphism is associated with susceptibility to preterm birth. BMC Medical Genetics, 16:63.

[15] Loosfelt, H., Atger, M., Misrahi, M., Guiochonmantel, A., and Logeat, F. (1986). Cloning and sequence-analysis of rabbit progesterone receptor complementary-DNA. *Proc Natl Acad Sci*, 83(23), 9045–9049.

[16] Lydon, J.P., DeMayo, F.J., Funk, C.R., Mani, S.K., Hughes, A.R., Montgomery, C.A., Shyamala, G., Conneely, O.M., and O'Malley, B.W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev*, 9, 2266–2278.

[17] Misrahi, M., Atger, M., and D'Auriol, L. (1987). Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. *Biochem Biophy Res Commun*, 143, 740-748.

[18] Mitra, M., Taraphder, S., Sonawane, G.S., and Verma, A. (2012). Nucleotide Sequencing and SNP Detection of Toll-Like Receptor-4 Gene in Murrah Buffalo (*Bubalus bubalis*). *ISRN Mol Biol*,1-7.

[19] Navani, N., Jain, P.K., Gupta, S., Sisodia, B., and Kumar, S. (2002). A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*). *Anim Genet*, 33, 149-154.

[20] Naylor, S.L., Helen-Devis, D., Hughes, M.R., O'Malley, B., and Lalley, P.A. (1989). The progesterone receptor gene is on mouse chromosome 9. *Cytogenet Cell Genet*, 51, 1051.

[21] Oswin perera, B.M. (1999). Reproduction in water buffalo: comparative aspects and implications for management. *J Reprod Fertil Supplement*, 54, 157-168.

[22] Peiro, R., Merchan, M., Santacreu, M.A., Argente, M.J., Garcia, M,L., Folch, J.M., and Blasco, A. (2008). Identification of single-nucleotide polymorphism in the progesterone receptor gene and its association with reproductive traits in rabbits. *Genetics*,180 (3), 1699-1705.

[23] Romano, A., Delvoux, B., Fischer, D.C., and Groothuis, P. (2007). The PROGINS polymorphism of the human progesterone receptor diminishes the response to progesterone. *J Mol Endocrinol*, 38 (1-2), 331-50.

[24] Rosati, A., and Van Vleck, L.D. (2002). Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for the Italian river buffalo *Bubalus bubalis* population. *Livest Prod Sci*, 74, 185–190.

[25] Rousseau-Merck, M.F., Misrahi, M., Loosfelt, H., Milgrom, E., and Berger, R. (1987). Localization of the human progesterone receptor gene to chromosome 11q22-q23. *Human Genet*, 77, 280-282.

[26] Sambrook, J., and Russel, D.W. (2001). Molecular Cloning: a laboratory manual. 3rd edn. Cold Spring Harbour Laboratory, New York, USA, 999.

[27] Schott, D.R., Shyamala, G., Schneider, W., and Perry, G. (1991). Molecular cloning, sequence analysis and expression of complementary DNA encoding murine progesterone receptor. *Biochemistry*, 30, 7014-7020.

[28] Sethi, R.K. (2003). Improving riverine and swamp buffaloes through breeding. Proc. of the Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb., 51-60.

[29] Shyamala, G., Schneider, W., and Schott, D. (1990). Developmental regulation of murine mammary progesterone receptor gene expression. *Endocrinology*, 126, 2882–2889.

[30] Suwattana, D., Jeorg, H., Reider, S., Chavananikul, V., and Stranzinger, G. (1999). A sequence of progesterone receptor homologue in fresh water crocodile (*Crocodylus siamensis*). *J Anim Breed Genet*, 116, 243-252.

[31] Vang, A., Salem, K. And Fowler, A.M. (2023). Progesterone Receptor Gene Polymorphisms and Breast Cancer Risk. Endocrinology, 164(4).