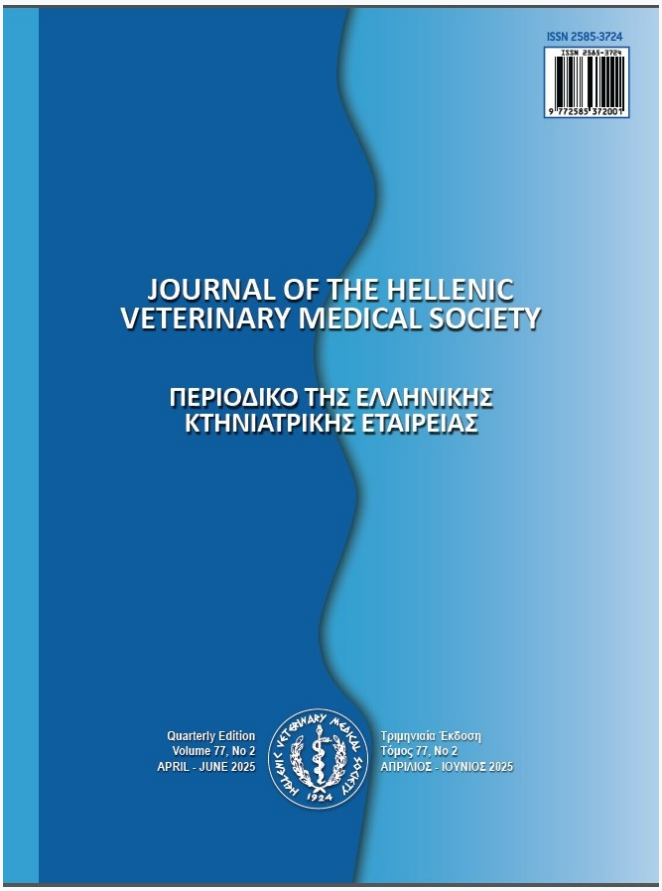
Journal of the Hellenic Veterinary Medical Society

Vol 76, No 2 (2025)



New insights into polymorphisms in candidate genes associated with incidence to repeat breeder in Italian buffaloes (Bubalus bubalis)

A Ateya, FA Safhi , AR Alhimaidi , AA Ammari , RA Amran , B Marghani , SI Rakha , S Sakr, H El-Emam

doi: 10.12681/jhvms.36239

To cite this article:

Ateya, A., Safhi , F., Alhimaidi , A., Ammari , A., Amran , R., Marghani , B., Rakha , S., Sakr, S., & El-Emam , H. (2025). New insights into polymorphisms in candidate genes associated with incidence to repeat breeder in Italian buffaloes (Bubalus bubalis). *Journal of the Hellenic Veterinary Medical Society*, *76*(2), 9055–9072. https://doi.org/10.12681/jhvms.36239

J HELLENIC VET MED SOC 2025, 77 (2): 9055-9072
ΠΕΚΕ 2025, 77 (2): 9055-9072

Research article
Ερευνητικό άρθρο

New insights into polymorphisms incandidate genes associated with incidence to repeat breeder in Italian buffaloes (*Bubalus bubalis*)

Ahmed Ateya,[1]* Fatmah A. Safhi,[2] Ahmad R. Alhimaidi,[3] Aiman A. Ammari,[3] Ramzi A. Amran,[3] Basma Marghani,[4,5] Shimaa I. Rakha,[6] Shimaa A. Sakr[1] and Huda El-Emam[1]

[1]*Department of Development of Animal Wealth, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.*

[2]*Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.*

[3]*Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia*

[4]*Department of Physiology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.*

[5]*Department of Biochemistry, Physiology, and Pharmacology, Faculty of Veterinary Medicine, King Salman International University, Egypt.*

[6]*Department of Theriogenology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.*

ABSTRACT: This study looked at the reproductive genes that could interact with repeat breeder incidence in Italian buffaloes. Two hundred and forty female Italian buffaloes (120 repeat breeder and 120 apparently normal) were used. Blood samples were collected from each buffalo into tubes encompassingEDTA anticoagulantto isolate DNA. PCR-DNA sequencing elicited nucleotide sequence differences between normal and repeat breeder buffaloes for the reproductive (*MFSD14A, PALMD, VPS13B, BMP, MTNR1A, TSHR, CSGALNACT1, CADM2, ZNF503, KRR1, MTPN, IGFBP7, LEP,*and *ABCC4*) genes. The likelihood of dissemination of all notable nucleotide variations varied noticeably between buffalo groups with and without repeat breeders, depending on Fisher's exact test ($p< 0.01$).All of the reproductive markers under research had the exonic region mutations in repeat breeder buffaloes versus normal ones.The results may confirm the importance of these markers' nucleotide variations as candidatesfor repeat breeder occurrence and offer a practical buffalo management strategy.

Keyword: Italian buffaloes; candidate gene; repeat breeder.

Correspondence author:
Ahmed Ateya
Department of Development of Animal Wealth, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt
dr_ahmedismail@mans.edu.eg; ahmed_ismail888@yahoo.com

Date of initial submission: **18-12-2023**
Date of acceptance: **18-11-2024**

INTRODUCTION

Economically important for long-term food production are reproductive features, especially in monotocous animals like cattle and buffalo (Shao et al., 2021). Repeat breeding, or an extended interval between two calves, may be indicative of poor reproduction or sterility. More inseminations, veterinary care, and hormone therapies are required to address this illness, all of which have an impact on current and upcoming lactations (Biochard, 1990). Additionally, extra expenses are spent due to the culling and replacement of animals with reproduction problems (Roxström & Strandberg, 2002). The best option for reducing culling expenses, maintaining significant genetic features, and boosting farm profits is fertility improvement (Dekkers, 1991). With relation to statistical distribution, binary, interval, and continuous attributes have previously been used to classify reproductive characteristics (Berry & Evans 2014). Reproductive characteristics have been classified to make them simpler for understanding and utilizing in livestock and breeding programs (Cammack et al., 2009). These categories include features relating to ovulation, mating, and calving.

Because buffalo are mono-ovulatory, only one follicle typically grows and ovulates during the oestrus phase, which significantly reduces fertility and limits the growth of the buffalo business (Li et al., 2018). The reproductive activity of the buffaloes follows a seasonal pattern, with compromised activity in the summer and regular reproductive cyclicity and conception in the autumn and winter (Das &, Khan 2010). Untangling the reproductive endocrinology of buffaloes will help researchers determine the cause of this reproductive restriction (Warriach et al., 2015). Quantitative features that affect reproduction are influenced by a variety of circumstances. Repeat breeding's occurrence in dairy farms can be reduced, and with it, the associated financial loss, by being aware of its underlying causes (Regmi&Dhakal, 2020). Repeat breeding can be caused by a wide range of factors, including insufficient fertilization, premature embryonic death, genital tract congenital or genetic faults, spermatozoa, ova or first zygote defects, microbial or traumatic inflammation, endocrine/hormonal problems, and management flaws (such as failing to identify estrus) (Yusuf et al., 2010).It is anticipated that genetic variants will influence gene expression, which will change the amount of one or more proteins, changing the phenotype of attributes (Musunuru et al., 2010;Lappalainen et al.,2013).A way to locate inherited components which affect susceptibility for common economic problems is marker-assisted selection (MAS) (Ashwell et al., 1997; Bishop et al., 1995). In the era of genomic assortment, excellent cow training groups that combine attributes with high-throughput genomic SNP marker data allow the use of selection strategies for emerging functional characteristics, such disease resistance (Buch et al., 2012).Another significant advantage of genetic selection is shortening of generational intervals (Schaeffer, 2006). A novel understanding of the identity of underlying genes and change in the genetic materialand how they might affect the characteristic has been claimed to be gained by integrating genetic markers to find significant genes linked with target traits (Li et al., 2014). The term "transcriptome" denotes all the genes in the genome that are accurately and at high throughput during particular physiological and pathological states (Kukurba& Montgomery, 2015).

Recent genome-wide association analysis studies have focused on novel genes related to bovine reproductive performance; however, no studies have yet looked at the relationship between the SNPs in these genes and the frequency of repeat breeders (Li et al., 2018; Mahrouset al., 2022). It is important to note that more research is necessary to follow potential symptoms that can imply reproductive failure from repeat breeder because there is limited knowledge on the reproduction pathways that determine buffalo resistance to or sensitivity to repeat breeder (Li et al.,2018). The molecular alterations may also help to identify repeat breeders and provide crucial information about the interactions between the physiologies of the various reproductive pathways (Sammad et al.,2022; Heidari et al.,2019). The effectiveness of putative reproductive (*MFSD14A, PALMD, VPS13B, BMP, MTNR1A, TSHR, CSGALNACT1, CADM2, ZNF503, KRR1, MTPN, IGFBP7, LEP,*and *ABCC4*) genes as candidates for repeat breeder incidence prediction and tracking in Italian buffaloes was examined in this study employing PCR-DNA sequencing methods.

MATERIALS AND METHODS

Dairy buffaloes and reseach samples

The current study was carried out on 240 pure Italian buffaloes aged 4.5 years. Buffaloes upraised on a private farm in the province of Delta region of northern Egypt of which 120 were repeat breeder and 120 appeared to be normal. Based on breeding history and clinical indications seen by skilled vets,

both groups were selected. All of the animals were raised in stalls with free access to water. It was determined that none of the study animals had ovarian pathologies like ovarian cysts or uterine, cervical, or vaginal inflammation or infection. The buffalo cows in the control group appeared to be in good condition and had a regular estrous cycle, nonetheless, no pregnancies were achieved in the repeat breeder groupnotwithstanding at least three successful attempts to artificially inseminate or mate with a bull in estrus (Devkota et al., 2022). Visual observation was used to identify estrus in both groups twice daily in the morning and in the evening. All animals were checked for foetal membrane slip and/or fremitus, and rectal palpation was used to confirm pregnancy diagnoses.

Five milliliters of blood were obtained by puncturing the jugular vein of each buffalo. To obtain whole blood and recover DNA samples were placed in tubes containing EDTA as anticoagulant. The Ethical Committee approved the sample collection and animal care techniques utilized in this study, and they complied with Mansoura University's regulations. The Mansoura University Animal Care and Use Committee (MU-ACUC) gave its approval to the study's protocol (code VM.R.23.11.26).

DNA extraction and PCR amplification

DNA from the genome was extracted using total blood by means of the genetic material JET full blood genomic DNA isolation kit and the producer's directions (Thermo scientific, Vilnius, Lithuania). Using Nanodrop, DNA of good purity and concentration was considered. The following reproductive (*MFSD14A, PALMD, VPS13B, BMP, MTNR1A, TSHR, CSGALNACT1, CADM2, ZNF503, KRR1, MTPN, IGFBP7, LEP,*and *ABCC4*) genes' parts of coding sites (CDS) were amplified. The oligonucleotide sequences for amplification were constructed using the PubMed *Bubalus bubalis* genome. Table 1 provides the primers utilized in the PCR.

The polymerase chain cloning mixture had been treated in a thermal cycler with a final volume of 50µL. The succeeding elements were present in every reaction container: 19microlitersd.d. water, 4 microliters of genetic material, 1 microliters of each primer pair, and 25 microliters of the master combination (Jena Bioscience, Jena, Germany). The PCR combinations remained in use for four minutes at a starting temperature of 95 °C for unwinding. The 34 cycles included one-minute denaturation rounds at 95 °C, one-minute annealing cycles based on the Table 1 temperature range, and 30-second for elongationat 72 °C, followed by ten minutes of final elongation. The sampleswere retained at 4 °C. Using agarose gel electrophoresis to obtain demonstrable outcomes and viewing PCR segment configurations underneath UV light, a gel certification technique was used.

Discoveringpolymorphism

Before DNA sequencing, Hamburg, Germany-based Jena Bioscience # pp-201s/Munich provided methods to purify PCR for getting rid of primer dimmers, non-specific bands, and other impurities and create the predicted scope's planned amplified product(-Boom et al.,1990). Using a Nanodrop (Waltham, Massachusetts, USA, UV-Vis spectrometer Q5000), adequate quality and good concentrations were obtained while measuring PCR output (Boesenberg-Smith et al., 2012). Sequence analysis of the PCR-produced amplification results has been utilized to find SNPs using normal and repeat breeder buffaloes. The PCR yields were sequenced using the Sanger et al., (1977) outlined enzyme chain terminator technique on an ABI 3730XL DNA sequencer (United States: Applied Biosystems, Waltham).

Chromas 1.45 and BLAST 2.0, the tools to evaluate results of DNA analysis (Altschul et al., 1990). Comparing the reproductive gene PCR results to the GenBank-provided reference gene sequences has revealed polymorphisms. The MEGA6 tool can detect differences in the amino acid categorizations among the examined genes according to sequence matching among the buffaloes under investigation (Tamura et al.,2007).

Statistical analysis

H_o:Polymorphisms of reproductive genes could not interact in the incidence to repeat breeder in Italian buffaloes.

H_A: Polymorphisms of reproductive genes could interact in the incidence to repeat breeder in Italian buffaloes.

Observing the significant distribution of SNPs for the discovered genes between the examined buffaloes was conducted using Fisher's exact test analysis($p < 0.01$). Graphpad statistical software was used to conduct the statistical analysis (Graphpad prism for Windows version 5.1, Graphpad software, Inc., San Diego, CA, USA).

Table 1. Oligonucleotide primers for reproductive genes used to study genetic polymorphisms.

Investigated marker	Sense	Antisense	Accession number	Annealing Temperature (°C)	Size of PCR product (bp)
MFSD14A	5′-GCGAACCGCAGTATCATGCTG-3′	5′-CCAGGCCATACGCCATACTTC-3′	XM_055584984.1	60	457
PALMD	5′-TGGCTTCTAGATGGAATCAGC-3′	5′-CTATTCCTTCATTTCTTTCC-3′	XM_006045619.1	58	343
VPS13B	5′-AATGAGCTATGTGAATCGCT-3′	5′- ACAACTCGTCTGATCAGACTCT-3′	XM_055546577.1	60	414
BMP	5′-ATGGTCCTTCTGAGCATCCTTA-3′	5′-GTGGAGCCTCTTCCTGAAGAAG-3′	EF375880.1	60	480
MTNR1A	5′-GTGAGCCTGGCAGTTGCAGAC-3′	5′- CACTCTCCATCTGACCTGAAG-3′	MF173058.1	56	465
TSHR	5′-ATCCGCCTCTGGCACGCCTAC-3′	5′-TGGCATAGAGGAATGGATTGGC-3′	KC415275.1	58	442
CSGALNACT1	5′- CTGCTCGCCTGGGCCTCCCGG-3′	5′- CGGTGGCCTGCTTGACGCCGGC-3′	XM_025275645.3	58	385
CADM2	5′-CGCAGCGCCGTTCTCCGCTTC-3′	5′-GAACCATCTTATATCAGCTGCA-3′	XM_025278930.3	60	495
ZNF503	5′-TCTCCGCCAGGCTAACCGCCT-3′	5′- CTTATCCGAGCCGGGTTTGGAG-3′	XM_025285122.3	60	361
KRR1	5′-CCGACGTCGGAGAACCGAGATG-3′	5′- CTAATGATGATATATGGATCA -3′	XM_006048659.4	58	278
MTPN	5′-ATGTGCGACAAGGAGTTCATG-3′	5′- GGCACCAGTGACATTCCAGAT -3′	XM_044946911.2	60	266
IGFBP7	5′-AGCTGGGCGAGACCCGCGATGC-3′	5′- GACAGAAGCAATTTCACGCAG -3′	XM_025290001.3	56	380
LEP	5′-CAGTCCGTCTCCTCCAAACAGA-3′	5′- TCAGCACCCAGGACTGAGGTC -3′	AF387814.1	58	360
ABCC4	5′-GCAGAAGGCGAACTTCTGCTC-3′	5′- TATGGGTTGAACTACTCTGGTG -3′	XM_045162483.1	60	355

MFSD14A= Major facilitator superfamily domain containing 14A; *PALMD*= Palmdelphin; *VPS13B*= Vacuolar protein sorting 13 homolog B; *BMP*= Bone morphogeneic protein; *MTNR1A*= Melatonin receptor 1B; *TSHR*= Thyroid stimulating hormone receptor; *CSGALNACT1*= Chondroitin sulfate N-acetylgalactosaminyltransferase 1; *CADM2*= Cell adhesion molecule 2; *ZNF503*= Zinc finger protein 503; *KRR1* = Small subunit processome component homolog; *MTPN*= Myotrophin; *IGFBP7*= insulin like growth factor binding protein 7; *LEP*= Leptin; and *ABCC4*= ATP-binding cassette sub-family C member 4.

RESULTS

SNP variations in amplified DNA nucleotides associated with repeat breeder were found in the results of PCR-DNA sequencing on both normal and repeat breeder buffaloes for the MFSD14A (457-bp), PALMD (343-bp), VPS13B (414-bp), BMP (480-bp), MTNR1A (465-bp), TSHR (442-bp), CSGALNACT1 (385-bp), CADM2 (495-bp), ZNF503 (361-bp), KRR1 (278-bp), MTPN (266-bp), IGFBP7 (380-bp), LEP (360-bp),and ABCC4 (355-bp) genes. The DNA sequence differences between the sequences of reference genes obtained from GenBank and the reproductive indicators examined in the studied buffaloes were used to verify each SNP that has been discovered (Figures S1–S14).

Table 2 shows the dissemination of a single base variation as well as a type of inherited change for reproductive indicators in normal and repeat breeder buffaloes. The SNPs' Fisher's exact test analysis revealed considerably different occurrences of the investigated markers in the normal and repeat breeder buffaloes ($p < 0.01$). All of the reproductive markers under research had the exonic region mutations, which resulted in different coding DNA sequences in repeat breeder buffaloes versus normal ones. Thirty SNPs were discovered using DNA sequencing of reproductive genes; 16 of them are synonymous and 15 is non-synonymous. The amino acid variations between the reproductive genes in all the research buffaloes and the reference sequences retrieved in GenBank were used to validate all observed SNPs and the corresponding amino acids (Figures S15–S28).

For theMFSD14Agene (457-bp), two observed recurrent synonymous SNPs, 165CT and 322TC, resulted in 55C and 108G respectively. One recurrent SNPs were found when the PALMDgene (343-bp) was sequenced. Synonymous mutation 36L occurred as a result of 108TC SNP. 175TC and 223TC involved a non-synonymous mutation, resulting in the substitution of the amino acid C59R, and Y75H respectively; were found in DNA sequences of the VPS13B gene (414-bp). The BMP gene (480-bp) contained three non-synonymous SNPs: 47TC, 80CA, and 184GA, which caused the amino acids V16A, P27Q, and V62I to be substituted, respectively. The nucleotide sequence of the MTNR1A gene (465-bp) revealed one recurrent synonymous SNP; 24N resulted from 72CT SNP. The 442-bp TSHR gene's DNA sequence revealed four identified

```
XM_055584984.1      GCGAACCGCAGTATCATGCTGGCCAAAAAGATCATCATTAAGGACGGAGGCACGCCTCAA      60
H                   GCGAACCGCAGTATCATGCTGGCCAAAAAGATCATCATTAAGGACGGAGGCACGCCTCAA      60
RR                  GCGAACCGCAGTATCATGCTGGCCAAAAAGATCATCATTAAGGACGGAGGCACGCCTCAA      60
                    ************************************************************

XM_055584984.1      GGAATAGGTTCTCCTAGTGTTTATCATGCAGTTATCGTCATCTTTTTGGAGTTTTTCGCT      120
H                   GGAATAGGTTCTCCTAGTGTTTATCATGCAGTTATCGTCATCTTTTTGGAGTTTTTCGCT      120
RR                  GGAATAGGTTCTCCTAGTGTTTATCATGCAGTTATCGTCATCTTTTTGGAGTTTTTCGCT      120
                    ************************************************************

XM_055584984.1      TGGGGATTATTGACAGCACCCACCTTGGTGGTATTACATGAAACCTTCCCTAAACATACA      180
H                   TGGGGATTATTGACAGCACCCACCTTGGTGGTATTACATGAAACCTTCCCTAAACATACA      180
RR                  TGGGGATTATTGACAGCACCCACCTTGGTGGTATTACATGAAACTTTCCCTAAACATACA      180
                    ******************************************* ***************

XM_055584984.1      TTTCTGATGAATGGCCTAATTCAAGGAGTAAAGGGTTTGTTGTCATTCCTCAGTGCCCCT      240
H                   TTTCTGATGAATGGCCTAATTCAAGGAGTAAAGGGTTTGTTGTCATTCCTCAGTGCCCCT      240
RR                  TTTCTGATGAATGGCCTAATTCAAGGAGTAAAGGGTTTGTTGTCATTCCTCAGTGCCCCT      240
                    ************************************************************

XM_055584984.1      CTTATTGGTGCTCTTTCTGATGTTTGGGGCCGAAAATCCTTCTTGCTGCTAACAGTATTT      300
H                   CTTATTGGTGCTCTTTCTGATGTTTGGGGCCGAAAATCCTTCTTGCTGCTAACAGTATTT      300
RR                  CTTATTGGTGCTCTTTCTGATGTTTGGGGCCGAAAATCCTTCTTGCTGCTAACAGTATTT      300
                    ************************************************************

XM_055584984.1      TTCACGTGTGCCCCAATTCCTTTAATGAAAATCAGCCCATGGTGGTACTTTGCTGTTATC      360
H                   TTCACGTGTGCCCCAATTCCTTTAATGAAAATCAGCCCATGGTGGTACTTTGCTGTTATC      360
RR                  TTCACGTGTGCCCCAATTCCTCTAATGAAAATCAGCCCATGGTGGTACTTTGCTGTTATC      360
                    ********************* ****************************************

XM_055584984.1      TCTGTTTCTGGGGTTTTTGCAGTGACTTTCTCCGTGGTATTTGCATATGTAGCAGATATA      420
H                   TCTGTTTCTGGGGTTTTTGCAGTGACTTTCTCCGTGGTATTTGCATATGTAGCAGATATA      420
RR                  TCTGTTTCTGGGGTTTTTGCAGTGACTTTCTCCGTGGTATTTGCATATGTAGCAGATATA      420
                    ************************************************************

XM_055584984.1      ACCCAAGAACATGAAAGAAGTATGGCGTATGGCCTGG 457
H                   ACCCAAGAACATGAAAGAAGTATGGCGTATGGCCTGG 457
RR                  ACCCAAGAACATGAAAGAAGTATGGCGTATGGCCTGG 457
                    ************************************
```

Figure S1. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_055584984.1| and *MFSD14A* marker (457-bp) sequences.

```
XM_006045619.4      TGGCTTCTAGATGGAATCAGCAGTGGAAAAGAGCAGGAAGAGATGAAGAAGCAAAATCAA      60
H                   TGGCTTCTAGATGGAATCAGCAGTGGAAAAGAGCAGGAAGAGATGAAGAAGCAAAATCAA      60
RR                  TGGCTTCTAGATGGAATCAGCAGTGGAAAAGAGCAGGAAGAGATGAAGAAGCAAAATCAA      60
                    ************************************************************

XM_006045619.4      CAAGACCAGCACCAGATCCAGGTTCTAGAACAAAGTATCCTCAGACTTGAGAAAGAGATC      120
H                   CAAGACCAGCACCAGATCCAGGTTCTAGAACAAAGTATCCTCAGACTCGAGAAAGAGATC      120
RR                  CAAGACCAGCACCAGATCCAGGTTCTAGAACAAAGTATCCTCAGACTTGAGAAAGAGATC      120
                    ********************************************** ************

XM_006045619.4      CAAGATCTTGAAAAGGCTGAACTGCAAATCTCAACCAATGAAGAAGCAATTTTAAAGAAA      180
H                   CAAGATCTTGAAAAGGCTGAACTGCAAATCTCAACCAATGAAGAAGCAATTTTAAAGAAA      180
RR                  CAAGATCTTGAAAAGGCTGAACTGCAAATCTCAACCAATGAAGAAGCAATTTTAAAGAAA      180
                    ************************************************************

XM_006045619.4      CTGAAATCAGTTGAGAGGACAACAGAAGACATAATAAGGTCTGTGAAGGTGGAAAAGGAA      240
H                   CTGAAATCAGTTGAGAGGACAACAGAAGACATAATAAGGTCTGTGAAGGTGGAAAAGGAA      240
RR                  CTGAAATCAGTTGAGAGGACAACAGAAGACATAATAAGGTCTGTGAAGGTGGAAAAGGAA      240
                    ************************************************************

XM_006045619.4      GAAACATCAGGAGAGTCAGTTGAAGACATCTATGCTAATATCCCCGACCTTCCAAAATCC      300
H                   GAAACATCAGGAGAGTCAGTTGAAGACATCTATGCTAATATCCCCGACCTTCCAAAATCC      300
RR                  GAAACATCAGGAGAGTCAGTTGAAGACATCTATGCTAATATCCCCGACCTTCCAAAATCC      300
                    ************************************************************

XM_006045619.4      TACATACCTTCCAGGTTAAGGAAGGAAAGAAATGAAGGAATAG    343
H                   TACATACCTTCCAGGTTAAGGAAGGAAAGAAATGAAGGAATAG    343
RR                  TACATACCTTCCAGGTTAAGGAAGGAAAGAAATGAAGGAATAG    343
                    *******************************************
```

Figure S2. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb| XM_006045619.4| and *PALMD* marker (343-bp) sequences.

```
XM_055546577.1      AATGAGCTATGTGAATCGCTACATCAAGAACTTAAAGCCGTCAGATCTACAGCTTTCGCT      60
H                   AATGAGCTATGTGAATCGCTACATCAAGAACTTAAAGCCGTCAGATCTACAGCTTTCGCT      60
RR                  AATGAGCTATGTGAATCGCTACATCAAGAACTTAAAGCCGTCAGATCTACAGCTTTCGCT      60
                    ************************************************************

XM_055546577.1      ATGGGGTGGAGACGTGGTTCTCAGCAAGCTGGAGTTAAAATTGGATGTGCTGGAGCAGGA      120
H                   ATGGGGTGGAGACGTGGTTCTCAGCAAGCTGGAGTTAAAATTGGATGTGCTGGAGCAGGA      120
RR                  ATGGGGTGGAGACGTGGTTCTCAGCAAGCTGGAGTTAAAATTGGATGTGCTGGAGCAGGA      120
                    ************************************************************

XM_055546577.1      ATTGAAATTACCATTCACTTTTTTAAGTGGACATATTCATGAACTGAGGATCCATGTACC      180
H                   ATTGAAATTACCATTCACTTTTTTAAGTGGACATATTCATGAACTGAGGATCCACGTACC      180
RR                  ATTGAAATTACCATTCACTTTTTTAAGTGGACATATTCATGAACTGAGGATCCATGTACC      180
                    ************************************************** *****

XM_055546577.1      ATGGACAAAACTGGGTTCAGAACCAGTGGTAATTACCATCAATACAATGGAATGCATTTT      240
H                   ATGGACAAAACTGGGTTCAGAACCAGTGGTAATTACCATCAATACAATGGAATGCATTTT      240
RR                  ATGGACAAAACTGGGTTCAGAACCAGTGGTAATTACCATCAACACAATGGAATGCATTTT      240
                    ****************************************** ****************

XM_055546577.1      GAAACTTAAAGATGGAATACAGGATGATCATGAAAGCTGTGGTTCTAATTCTACCAACCG      300
H                   GAAACTTAAAGATGGAATACAGGATGATCATGAAAGCTGTGGTTCTAATTCTACCAACCG      300
RR                  GAAACTTAAAGATGGAATACAGGATGATCATGAAAGCTGTGGTTCTAATTCTACCAACCG      300
                    ************************************************************

XM_055546577.1      TAGTACTACTGAGAACACAAAAACATCAGCAAAACCCCGAAGAATTCAACAGGCCACTCC      360
H                   TAGTACTACTGAGAACACAAAAACATCAGCAAAACCCCGAAGAATTCAACAGGCCACTCC      360
RR                  TAGTACTACTGAGAACACAAAAACATCAGCAAAACCCCGAAGAATTCAACAGGCCACTCC      360
                    ************************************************************

XM_055546577.1      CACAGATCCTGATTTGCCACCAGGCTATGTACAGAGTCTGATCAGACGAGTTGT   414
H                   CACAGATCCTGATTTGCCACCAGGCTATGTACAGAGTCTGATCAGACGAGTTGT   414
RR                  CACAGATCCTGATTTGCCACCAGGCTATGTACAGAGTCTGATCAGACGAGTTGT   414
                    ********************************************************
```

Figure S3. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_055546577.1| and *VPS13B* marker (414-bp) sequences.

frequent synonymous SNPs; 87CT, 126TC, 177CT, and 372CT, which were associated with the amino acids 29S, 42T, 59I, 42S and 124V.

For the CSGALNACT1gene (385-bp), one synonymous SNP was found; the results of 135GAwas45T. Three recurrent synonymous SNPs; 105GA, 168TC, and 354GT cleared during DNA sequencing of the CADM2 (495-bp) gene, which resulted in the amino acids 50R, 56N, and 118T, respectively.One recurrent non-synonymous SNP, 217GC, was elaborated using DNA sequencing of the ZNF503 gene (361-bp), which led to the substitution of the amino acid

```
EF375880.1      ATGGTCCTTCTGAGCATCCTTAGAATCCTTCTTCTTTGGGGACTGGTGCTTTTTATGGAA 60
H               ATGGTCCTTCTGAGCATCCTTAGAATCCTTCTTCTTTGGGGACTGGTGCTTTTTATGGAA 60
RR              ATGGTCCTTCTGAGCATCCTTAGAATCCTTCTTCTTTGGGGACTGGCGCTTTTTATGGAA 60
                ************************************************ ************

EF375880.1      CATAGGGTCCAAATGACACCGGTAGGGCAGCCCTCTATTGCCCACCTGCCTGAGGCCCCT 120
H               CATAGGGTCCAAATGACACAGGTAGGGCAGCCCTCTATTGCCCACCTGCCTGAGGCCCCT 120
RR              CATAGGGTCCAAATGACACCGGTAGGGCAGCCCTCTATTGCCCACCTGCCTGAGGCCCCT 120
                ****************** ****************************************

EF375880.1      ACCTTGCCCCTGATTCAGGAGCTGCTAGAAGAAGCCCCTGGCAAGCTGCAGAGGAAGCCG 180
H               ACCTTGCCCCTGATTCAGGAGCTGCTAGAAGAAGCCCCTGGCAAGCTGCAGAGGAAGCCG 180
RR              ACCTTGCCCCTGATTCAGGAGCTGCTAGAAGAAGCCCCTGGCAAGCTGCAGAGGAAGCCG 180
                ************************************************************

EF375880.1      CGGGTCTTAGGGCATCCCTTACGGTATATGCTGGAGTTGTACCACCGTTCAGCTGACGCA 240
H               CGGATCTTAGGGCATCCCTTACGGTATATGCTGGAGTTGTACCACCGTTCAGCTGACGCA 240
RR              CGGGTCTTAGGGCATCCCTTACGGTATATGCTGGAGTTGTACCACCGTTCAGCTGACGCA 240
                *** ************************************************************

EF375880.1      AGTGGACACCCTAGGGAAAACCGCACCATTGGGGCCACCATGGTGAGGCTGGTGAGGCCA 300
H               AGTGGACACCCTAGGGAAAACCGCACCATTGGGGCCACCATGGTGAGGCTGGTGAGGCCA 300
RR              AGTGGACACCCTAGGGAAAACCGCACCATTGGGGCCACCATGGTGAGGCTGGTGAGGCCA 300
                ************************************************************

EF375880.1      CTGGCTAGTGTAGCAAGGCCTCTCAGAGGCTCCTGGCACATACAGACCCTGGACTTTCCT 360
H               CTGGCTAGTGTAGCAAGGCCTCTCAGAGGCTCCTGGCACATACAGACCCTGGACTTTCCT 360
RR              CTGGCTAGTGTAGCAAGGCCTCTCAGAGGCTCCTGGCACATACAGACCCTGGACTTTCCT 360
                ************************************************************

EF375880.1      CTGAGACCAAACCGGGTAGCATACCAACTAGTCAGAGCCACTGTGGTTTACCGCCATCAA 420
H               CTGAGACCAAACCGGGTAGCATACCAACTAGTCAGAGCCACTGTGGTTTACCGCCATCAA 420
RR              CTGAGACCAAACCGGGTAGCATACCAACTAGTCAGAGCCACTGTGGTTTACCGCCATCAG 420
                ************************************************************

EF375880.1      CTTCACCTAACTCATTCCCACCTCTCCTGCCATGTGGAGCCCTGGGTCCAGAAAAGCCCA 480
H               CTTCACCTAACTCATTCCCACCTCTCCTGCCATGTGGAGCCCTGGGTCCAGAAAAGCCCA 480
RR              CTTCACCTAACTCATTCCCACCTCTCCTGCCATGTGGAGCCCTGGGTCCAGAAAAGCCCA 480
                ************************************************************
```

Figure S4. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|EF375880.1| and *BMP* marker (480-bp) sequences.

```
MF173058.1      GTGAGCCTGGCAGTTGCAGACCTGCTGGTGGCCGTGTATCCGTACCCCTTGGCGCTGGCG 60
H               GTGAGCCTGGCAGTTGCAGACCTGCTGGTGGCCGTGTATCCGTACCCCTTGGCGCTGGCG 60
RR              GTGAGCCTGGCAGTTGCAGACCTGCTGGTGGCCGTGTATCCGTACCCCTTGGCGCTGGCG 60
                ************************************************************

MF173058.1      TCTATAGTTAACGATGGGTGGAGCCTGAGCTCCCTGCATTGCCAACTTAGTGGCTTCCTG 120
H               TCTATAGTTAATGATGGGTGGAGCCTGAGCTCCCTGCATTGCCAACTTAGTGGCTTCCTG 120
RR              TCTATAGTTAACGATGGGTGGAGCCTGAGCTCCCTGCATTGCCAACTTAGTGGCTTCCTG 120
                *********** ************************************************

MF173058.1      ATGGGCTTGAGCGTCATCGGGTCCGTTTTCAATATCACGGGAATTGCCATCAACCGCTAT 180
H               ATGGGCTTGAGCGTCATCGGGTCCGTTTTCAATATCACGGGAATTGCCATCAACCGCTAT 180
RR              ATGGGCTTGAGCGTCATCGGGTCCGTTTTCAATATCACGGGAATTGCCATCAACCGCTAT 180
                ************************************************************

MF173058.1      TGCTGCATCTGCCACAGCCTCAGATACGACAAGCTGTATAGCAGCACAAATTCCCTCTGC 240
H               TGCTGCATCTGCCACAGCCTCAGATACGACAAGCTGTATAGCAGCACAAATTCCCTCTGC 240
RR              TGCTGCATCTGCCACAGCCTCAGATACGACAAGCTGTATAGCAGCACAAATTCCCTCTGC 240
                ************************************************************

MF173058.1      TACGTGTTCCTGATATGGCTGCTGACGTTCGTGGCGATCGTGCCCAACCTGTGTGTGGGG 300
H               TACGTGTTCCTGATATGGCTGCTGACGTTCGTGGCGATCGTGCCCAACCTGTGTGTGGGG 300
RR              TACGTGTTCCTGATATGGCTGCTGACGTTCGTGGCGATCGTGCCCAACCTGTGTGTGGGG 300
                ************************************************************

MF173058.1      ACCCTGCGGTACGACCCGAGGATCTATTCTTGTACCTTCACACAGTCCGTCAGCTCAGCC 360
H               ACCCTGCGGTACGACCCGAGGATCTATTCTTGTACCTTCACACAGTCCGTCAGCTCAGCC 360
RR              ACCCTGCGGTACGACCCGAGGATCTATTCTTGTACCTTCACACAGTCCGTCAGCTCAGCC 360
                ************************************************************

MF173058.1      TACACGATCGCCGTGGTGGTGTTCCATTTCATAGTTCCAATGCTCGTAGTCATCTTCTGT 420
H               TACACGATCGCCGTGGTGGTGTTCCATTTCATAGTTCCAATGCTCGTAGTCATCTTCTGT 420
RR              TACACGATCGCCGTGGTGGTGTTCCATTTCATAGTTCCAATGCTCGTAGTCATCTTCTGT 420
                ************************************************************

MF173058.1      TACCTGAGAATCTGGGCCCTGGTTCTTCAGGTCAGATGGAGAGTG      465
H               TACCTGAGAATCTGGGCCCTGGTTCTTCAGGTCAGATGGAGAGTG      465
RR              TACCTGAGAATCTGGGCCCTGGTTCTTCAGGTCAGATGGAGAGTG      465
                *********************************************
```

Figure S5. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|MF173058.1| and *MTNR1A* marker (465-bp) sequences.

```
KC415275.1      ATCCGCCTCTGGCACGCCTACGTCATCATGCTGGGGGGCTGGGTTTGCTGCTTCCTGCTC 60
H               ATCCGCCTCTGGCACGCCTACGTCATCATGCTGGGGGGCTGGGTTTGCTGCTTCCTGCTC 60
RR              ATCCGCCTCTGGCACGCCTACGTCATCATGCTGGGGGGCTGGGTTTGCTGCTTCCTGCTC 60
                ************************************************************

KC415275.1      GCCCTGCTCCCTTTGGTGGGAATAAGCAGCTATGCCAAGGTCAGCATCTGCCTGCCCATG 120
H               GCCCTGCTCCCTTTGGTGGGAATAAGCAGCTATGCCAAGGTCAGCATCTGCCTGCCCATG 120
RR              GCCCTGCTCCCTTTGGTGGGAATAAGTAGCTATGCCAAGGTCAGCATCTGCCTGCCCATG 120
                ************************** ***********************************

KC415275.1      GACACTGAGACTCCTCTTGCCCTGGCGTACATTATCCTCGTTCTGTTACTCAACATCGTT 180
H               GACACTGAGACTCCTCTTGCCCTGGCGTACATTATCCTCGTTCTGTTACTCAACATTGTT 180
RR              GACACCGAGACTCCTCTTGCCCTGGCGTACATTATCCTCGTTCTGTTACTCAACATCGTT 180
                ***** ********************************************** ***

KC415275.1      GCCTTTATCATCGTCTGTGCCTGTTACGTGAAGATCTACATCACAGTCCGAAATCCCCAC 240
H               GCCTTTATCATCGTCTGTGCCTGTTACGTGAAGATCTACATCACAGTCCGAAATCCCCAC 240
RR              GCCTTTATCATCGTCTGTGCCTGTTACGTGAAGATCTACATCACAGTCCGAAATCCCCAC 240
                ************************************************************

KC415275.1      TACAACCCGGGGGACAAAGATACTAGAATTGCCAAAAGGATGGCTGTGTTGATCTTCACT 300
H               TACAACCCGGGGGACAAAGATACTAGAATTGCCAAAAGGATGGCTGTGTTGATCTTCACT 300
RR              TACAACCCGGGGGACAAAGATACTAGAATTGCCAAAAGGATGGCTGTGTTGATCTTCACT 300
                ************************************************************

KC415275.1      GACTTCATGTGCATGGCCCCAATCTCTTTCTACGCTCTGTCGGCCCTTATGAACAAGCCT 360
H               GACTTCATGTGCATGGCCCCAATCTCTTTCTACGCTCTGTCGGCCCTTATGAACAAGCCT 360
RR              GACTTCATGTGCATGGCCCCAATCTCTTTCTACGCTCTGTCGGCCCTTATGAACAAGCCT 360
                ************************************************************

KC415275.1      CTCATCACCGTCACCAATTCCAAAATCTTGCTGGTCCTCTTCTACCCACTTAACTCCTGT 420
H               CTCATCACCGTCACCAATTCCAAAATCTTGCTGGTCCTCTTCTACCCACTTAACTCCTGT 420
RR              CTCATCACCGTTACCAATTCCAAAATCTTGCTGGTCCTCTTCTACCCACTTAACTCCTGT 420
                *********** ************************************************

KC415275.1      GCCAATCCATTCCTCTATGCCA442
H               GCCAATCCATTCCTCTATGCCA442
RR              GCCAATCCATTCCTCTATGCCA442
                **********************
```

Figure S6. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|KC415275.1| and *TSHR* marker (442-bp) sequences.

```
XM_025275645.3      CTGCTCGCCTGGGCCTCCCGGGTGGTGGTCTTGCTCGTGCTTCTCTGCTGCGTGGCGTCT      60
H                   CTGCTCGCCTGGGCCTCCCGGGTGGTGGTCTTGCTCGTGCTTCTCTGCTGCGTGGCGTCT      60
RR                  CTGCTCGCCTGGGCCTCCCGGGTGGTGGTCTTGCTCGTGCTTCTCTGCTGCGTGGCGTCT      60
                    ************************************************************

XM_025275645.3      GTCCTCTACATGCTGGCCTGCACGCCGAGGGGCGATGATGAGCGCCAGGGGCTGCCCAGG      120
H                   GTCCTCTACATGCTGGCCTGCACGCCGAGGGGCGATGATGAGCGCCAGGGGCTGCCCAGG      120
RR                  GTCCTCTACATGCTGGCCTGCACGCCGAGGGGCGATGATGAGCGCCAGGGGCTGCCCAGG      120
                    ************************************************************

XM_025275645.3      GCCAACGGCCCCACGGGCAGGGCCGGCTCCCAGGCGGCGGTGCTCGGCTGGGAGGAGCGT      180
H                   GCCAACGGCCCCACAGGCAGGGCCGGCTCCCAGGCGGCGGTGCTCGGCTGGGAGGAGCGT      180
RR                  GCCAACGGCCCCACGGGCAGGGCCGGCTCCCAGGCGGCGGTGCTCGGCTGGGAGGAGCGT      180
                    ************** ************************************************

XM_025275645.3      CACCGCGACCACGTCGGGAGCCTCAAGAGGCAAATTGCGCAGCTGCAGGAGGAGCTGCAA      240
H                   CACCGCGACCACGTCGGGAGCCTCAAGAGGCAAATTGCGCAGCTGCAGGAGGAGCTGCAA      240
RR                  CACCGCGACCACGTCGGGAGCCTCAAGAGGCAAATTGCGCAGCTGCAGGAGGAGCTGCAA      240
                    ************************************************************

XM_025275645.3      GAGCGGAGCGAGCAGCTGAAGGCCGCGCAGCAGCTGGCGGGGGAGCCCGCGGGTGCCCCG      300
H                   GAGCGGAGCGAGCAGCTGAAGGCCGCGCAGCAGCTGGCGGGGGAGCCCGCGGGTGCCCCG      300
RR                  GAGCGGAGCGAGCAGCTGAGGGCCGCGCAGCAGCTGGCGGGGGAGCCCGCGGGTGCCCCG      300
                    ****************** ****************************************

XM_025275645.3      GGCAGGGCCCAGGCCGACCTGCTGGCCTTCCTGCGCTCGCAGGTGGACAGGGCGGAGGTG      360
H                   GGCAGGGCCCAGGCCGACCTGCTGGCCTTCCTGCGCTCGCAGGTGGACAGGGCGGAGGTG      360
RR                  GGCAGGGCCCAGGCCGACCTGCTGGCCTTCCTGCGCTCGCAGGTGGACAGGGCGGAGGTG      360
                    ************************************************************

XM_025275645.3      CACGCCGGCGTCAAGCAGGCCACCG   385
H                   CACGCCGGCGTCAAGCAGGCCACCG   385
RR                  CACGCCGGCGTCAAGCAGGCCACCG   385
                    *************************
```

Figure S7. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_025275645.3| and *CSGALNACT1* marker (385-bp) sequences.

```
XM_025278930.3      CGCAGCGCCGTTCTCCGCTTCTACAGTGTCTGCGGGCTCCTGCTACAAGCGGCTGCTTCA      60
H                   CGCAGCGCCGTTCTCCGCTTCTACAGTGTCTGCGGGCTCCTGCTACAAGCGGCTGCTTCA      60
RR                  CGCAGCGCCGTTCTCCGCTTCTACAGTGTCTGCGGGCTCCTGCTACAAGCGGCTGCTTCA      60
                    ************************************************************

XM_025278930.3      AAGAATAAAGTTAAAGGCAGCCAAGGGCAGTTTCCACTCACACAGAATGTAACGGTTGTT      120
H                   AAGAATAAAGTTAAAGGCAGCCAAGGGCAAATTTCCACTCACACAGAATGTAACGGTTGTT      120
RR                  AAGAATAAAGTTAAAGGCAGCCAAGGGCAGTTTCCACTCACACAGAATGTAACGGTTGTT      120
                    ****************************** ******************************

XM_025278930.3      GAAGGCGGAACTGCAATTTTGACCTGCAGGGTTGATCAAAATGATAATACCTCCCTCCAG      180
H                   GAAGGCGGAACTGCAATTTTGACCTGCAGGGTTGATCAAAATGATAACACCTCCCTCCAG      180
RR                  GAAGGCGGAACTGCAATTTTGACCTGCAGGGTTGATCAAAATGATAATACCTCCCTCCAG      180
                    ************************************************ ************

XM_025278930.3      TGGTCAAATCCAGCTCAACAGACTCTGTACTTTGATGACAAGAAAGCCTTAAGAGACAAT      240
H                   TGGTCAAATCCAGCTCAACAGACTCTGTACTTTGATGACAAGAAAGCCTTAAGAGACAAT      240
RR                  TGGTCAAATCCAGCTCAACAGACTCTGTACTTTGATGACAAGAAAGCCTTAAGAGACAAT      240
                    ************************************************************

XM_025278930.3      AGAATCGAGCTGGTTCGGGCTTCCTGGCATGAACTGAGTATCAGCGTCAGTGATGTCTCT      300
H                   AGAATCGAGCTGGTTCGGGCTTCCTGGCATGAACTGAGTATCAGCGTCAGTGATGTCTCT      300
RR                  AGAATCGAGCTGGTTCGGGCTTCCTGGCATGAACTGAGTATCAGCGTCAGTGATGTCTCT      300
                    ************************************************************

XM_025278930.3      CTGTCTGATGAAGGACAGTACACCTGTTCTTTATTTACAATGCCTGTCAAAACGTCCAAG      360
H                   CTGTCTGATGAAGGACAGTACACCTGTTCTTTATTTACAATGCCTGTCAAAACTTCCAAG      360
RR                  CTGTCTGATGAAGGACAGTACACCTGTTCTTTATTTACAATGCCTGTCAAAACGTCCAAG      360
                    ************************************************** ******

XM_025278930.3      GCCTATCTCACTGTCCTGGGTGTTCCTGAGAAACCTCAAATTAGTGGATTTTCATCCCCA      420
H                   GCCTATCTCACTGTCCTGGGTGTTCCTGAGAAACCTCAAATTAGTGGATTTTCATCCCCA      420
RR                  GCCTATCTCACTGTCCTGGGTGTTCCTGAGAAACCTCAAATTAGTGGATTTTCATCCCCA      420
                    ************************************************************

XM_025278930.3      GTTATGGAGGGAGACTTGATGCAGCTGACTTGTAAAACATCTGGTAGTAAACCTGCAGCT      480
H                   GTTATGGAGGGAGACTTGATGCAGCTGACTTGTAAAACATCTGGTAGTAAACCTGCAGCT      480
RR                  GTTATGGAGGGAGACTTGATGCAGCTGACTTGTAAAACATCTGGTAGTAAACCTGCAGCT      480
                    ************************************************************

XM_025278930.3      GATATAAGATGGTTC   495
H                   GATATAAGATGGTTC   495
RR                  GATATAAGATGGTTC   495
                    ***************
```

Figure S8. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_025278930.3| and *CADM2* marker (495-bp) sequences.

```
XM_025285122.3      TCTCCGCCAGGCTAACCGCCTGCCCATCAAGGTGCTGAAGATGCTGACGGCACGGACTGG      60
H                   TCTCCGCCAGGCTAACCGCCTGCCCATCAAGGTGCTGAAGATGCTGACGGCACGGACTGG      60
RR                  TCTCCGCCAGGCTAACCGCCTGCCCATCAAGGTGCTGAAGATGCTGACGGCACGGACTGG      60
                    ************************************************************

XM_025285122.3      CCATATTTTGCACCCTGAGTACCTGCAGCCCCTGCCTTCCACTCCCGTCAGCCCCATAGA      120
H                   CCATATTTTGCACCCTGAGTACCTGCAGCCCCTGCCTTCCACTCCCGTCAGCCCCATAGA      120
RR                  CCATATTTTGCACCCTGAGTACCTGCAGCCCCTGCCTTCCACTCCCGTCAGCCCCATAGA      120
                    ************************************************************

XM_025285122.3      GCTCGATGCCAAGAAGAGCCCGCTGGCGCTGTTGGCCCAAACATGCTCGCAGATCGGGAA      180
H                   GCTCGATGCCAAGAAGAGCCCGCTGGCGCTGTTGGCCCAAACATGCTCGCAGATCGGGAA      180
RR                  GCTCGATGCCAAGAAGAGCCCGCTGGCGCTGTTGGCCCAAACATGCTCGCAGATCGGGAA      180
                    ************************************************************

XM_025285122.3      GCCCGACCCCTCGCCCTCGTCCAAACTCTCCTCAGTGGCCTCCAACGGGGGTGGCACGGG      240
H                   GCCCGACCCCTCGCCCTCGTCCAAACTCTCCTCAGTGGCCTCCAACGGGGGTGGCACGGG      240
RR                  GCCCGACCCCTCGCCCTCGTCCAAACTCTCCTCAGTCGCCTCCAACGGGGGTGGCACGGG      240
                    ********************************** ************************

XM_025285122.3      CGGTGCCGGCGGCGGCGCCGGGGGCGACAAGGACGCCAAGTCGGGCCCCCTCAAGCTGAG      300
H                   CGGTGCCGGCGGCGGCGCCGGGGGCGACAAGGACGCCAAGTCGGGCCCCCTCAAGCTGAG      300
RR                  CGGTGCCGGCGGCGGCGCCGGGGGCGACAAGGACGCCAAGTCGGGCCCCCTCAAGCTGAG      300
                    ************************************************************

XM_025285122.3      CGACATCGGCGTGGAAGACAAGTCGAGTTTCAAGCCGTACTCCAAACCCGGCTCGGATAA      360
H                   CGACATCGGCGTGGAAGACAAGTCGAGTTTCAAGCCGTACTCCAAACCCGGCTCGGATAA      360
RR                  CGACATCGGCGTGGAAGACAAGTCGAGTTTCAAGCCGTACTCCAAACCCGGCTCGGATAA      360
                    ************************************************************

XM_025285122.3      G        361
H                   G        361
RR                  G        361
                    *
```

Figure S9. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_025285122.3| and *ZNF503* marker (361-bp) sequences**.**

```
XM_006048659.4      CCGACGTCGGAGAACCGAGATGAATCTGAACTCCTCACTGTTCCAGATGGTTGGAAGGAG      60
H                   CCGACGTCGGAGAACCGAGATGAATCTGAACTCCTCACTGTTCCAGATGGTTGGAAGGAG      60
RR                  CCGACGTCGGAGAACCGAGATGAATCTGAACTCCTCACTGTTCCAGATGGTTGGAAGGAG      60
                    ************************************************************

XM_006048659.4      CCAGCTTTCTCCAAAGAGGACAATCCCAGAGGACTTCTGGAGGAGAGCAGTTTTGCAACT      120
H                   CCAGCTTTCTCCAAAGAGGACAATCCCAGAGGACTTCTGGAGGAGAGCAGTTTTGCAACT      120
RR                  CCAGCTTTCTCCAAAGAGGACAATCCCAGAGGACTTCTGGAGGAGAGCAGTTTTGCAACT      120
                    ************************************************************

XM_006048659.4      TTGTTTCCGAAATACAGAGAGGCTTACTTGAAAGAGTGCTGGCCATTGGTACAGAAAGCC      180
H                   TTGTTTCCGAAATACAGAGAGGCTTACTTGAAAGAGTGCTGGCCATTAGTACAGAAAGCC      180
RR                  TTGTTTCCGAAATACAGAGAGGCTTACTTGAAAGAGTGCTGGCCATTGGTACAGAAAGCC      180
                    ************************************************ ************

XM_006048659.4      TTGAATGAACATCATGTTAATGCCACCCTGGACCTGATTGAGGGCAGCATGACTGTCTGC      240
H                   TTGAATGAACATCATGTTAATGCCACCCTGGACCTGATTGAGGGCAGCATGACTGTCTGC      240
RR                  TTGAATGAACATCATGTTAATGCCACCCTGGACCTGATTGAGGGCAGCATGACTGTCTGC      240
                    ************************************************************

XM_006048659.4      ACCACCAAGAAGACATTTGATCCATATATCATCATTAG          278
H                   ACCACCAAGAAGACATTTGATCCATATATCATCATTAG          278
RR                  ACCACCAAGAAGACATTTGATCCATATATCATCATTAG          278
                    ***************************************
```

Figure S10. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_006048659.4| and *KRR1* marker (278-bp) sequences.

```
XM_044946911.2      ATGTGCGACAAGGAGTTCATGTGGGCCCTGAAAAACGGAGACCTAGATGAGGTGAAAGAC      60
H                   ATGTGCGACAAGGAGTTCATGTGGGCCCTGAAAAACGGAGACCTGGATGAGGTGAAAGAC      60
RR                  ATGTGCGACAAGGAGTTCATGTGGGCCCTGAAAAACGGAGACCTAGATGAGGTGAAAGAC      60
                    ******************************************* ***************

XM_044946911.2      TATGTGGCCAAGGGAGAAGATGTCAACCGGACACTAGAAGGTGGAAGAAAGCCTCTTCAT      120
H                   TATGTGGCCAAGGGAGAAGATGTCAACCGGACACTAGAAGGTGGAAGAAAGCCTCTTCAT      120
RR                  TATGTGGCCAAGGGAGAAGATGTCAACCGGACACTAGAAGGTGGAAGAAAGCCTCTTCAT      120
                    ************************************************************

XM_044946911.2      TATGCAGCAGATTGTGGACAGCTTGAAATCCTGGAATTTCTGCTGCTGAAAGGAGCAGAT      180
H                   TATGCAGCAGATTGTGGACAGCTTGAAATCCTGGAATTTCTGCTGCTGAAAGGAGCAGAT      180
RR                  TATGCAGCAGATTGTGGACAGCTTGAAATCCTGGAATTTCTGCTGCTGAAAGGAGCAGAT      180
                    ************************************************************

XM_044946911.2      ATTAATGCTCCAGATAAACATCATATCACACCTCTTCTGTCTGCCGTCTATGAAGGTCAT      240
H                   ATTAATGCTCCAGATAAACATCATATCACACCTCTTCTGTCTGCCGTCTATGAAGGTCAT      240
RR                  ATTAATGCCCCAGATAAACATCATATCACACCTCTTCTGTCTGCCGTCTATGAAGGTCAT      240
                    ******** ***************************************************

XM_044946911.2      GTTTCCTGCGTGAAATTGCTTCTGTC  266
H                   GTTTCCTGCGTGAAATTGCTTCTGTC  266
RR                  GTTTCCTGCGTGAAATTGCTTCTGTC  266
                    *************************
```

Figure S11. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_044946911.2| and *MTPN* marker (266-bp) sequences.

G73R.The nucleotide sequence of the KRR1 gene (278-bp) revealed one synonymous SNP; 56A resulted from 168GA SNP. Two recurrent SNPs in the MTPN gene (266-bp) were discovered using DNA sequencing; 45AG and 189TC, resulted in synonymous mutations, 15L and 63A, respectively. Three recurrent non-synonymous SNPs were found in the IGFBP7 gene (380-bp); 38GC, 107TC, and 161AC changed the amino acids R13P, V36A and Q54P, respectively.Two recurrent synonymous SNPs, 93GA, and 216GA induced amino acids 31L, and 72P were found in the LEP gene (360-bp). Four recurrent SNPs in the ABCC4 gene (355-bp) were discovered using DNA sequencing; one of these, 294CT resulted in synonymous mutations, 98I. While the non-synonymous mutation brought about by the SNPs 65CT, 109AG and 306GT led to the substitution of the amino acids S22L, R37G, and L102F, respectively

DISCUSSION

Efficiency of investigated reproductive genes as candidates for repeat breeder incidence

For the effective exploitation of disease-resistant cattle or the complete eradication of ill animals, knowledge of the genes, it is necessary to understand the fundamental mutations and interactions that contribute to resistance(Pal& Chakravarty, 2020). The reproductive (*MFSD14A, PALMD, VPS13B, BMP,*

```
XM_025290001.3      AGCTGGGCGAGACCCGCGATGCATGCGGCTGCTGCCCGGTGTGCGCCCGCGGCGAGGGCG      60
H                   AGCTGGGCGAGACCCGCGATGCATGCGGCTGCTGCCCGGTGTGCGCCCGCGGCGAGGGCG      60
RR                  AGCTGGGCGAGACCCGCGATGCATGCGGCTGCTGCCCCGTGTGCGCCCGCGGCGAGGGCG      60
                    ************************************ ********************

XM_025290001.3      AGCCGTGCGGGGGTGGCGGCGCCGGCAGGGGGCACTGCGCGCCGGGTATGGAGTGCGTGA      120
H                   AGCCGTGCGGGGGTGGCGGCGCCGGCAGGGGGCACTGCGCGCCGGGTATGGAGTGCGTGA      120
RR                  AGCCGTGCGGGGGTGGCGGCGCCGGCAGGGGGCACTGCGCGCCGGGCATGGAGTGCGTGA      120
                    ********************************************** *************

XM_025290001.3      AGAGCCGCAAGAGGCGGAAGGGTAAAGCCGGGGCAGCAGCAGGCGGCCCGGTTGTGAGCG      180
H                   AGAGCCGCAAGAGGCGGAAGGGTAAAGCCGGGGCAGCAGCCGGCGGCCCGGTTGTGAGCG      180
RR                  AGAGCCGCAAGAGGCGGAAGGGTAAAGCCGGGGCAGCAGCAGGCGGCCCGGTTGTGAGCG      180
                    ************************************** ******************

XM_025290001.3      GCGTGTGTGTGTGCAAGAGCCGCTACCCCGTGTGCGGCAGCGACGGTGTCACCTACTCCA      240
H                   GCGTGTGTGTGTGCAAGAGCCGCTACCCCGTGTGCGGCAGCGACGGTGTCACCTACTCCA      240
RR                  GCGTGTGTGTGTGCAAGAGCCGCTACCCCGTGTGCGGCAGCGACGGTGTCACCTACTCCA      240
                    ************************************************************

XM_025290001.3      GCGGCTGCCAGCTGCGCGCCGCCAGCCTCAGGGCCGAGAGCCGCGGGGAGAAGGCCATCA      300
H                   GCGGCTGCCAGCTGCGCGCCGCCAGCCTCAGGGCCGAGAGCCGCGGGGAGAAGGCCATCA      300
RR                  GCGGCTGCCAGCTGCGCGCCGCCAGCCTCAGGGCCGAGAGCCGCGGGGAGAAGGCCATCA      300
                    ************************************************************

XM_025290001.3      CCCAGGTCAGCAAGGGCACCTGCGAGCAAGGTCCTTCCATCGTGACACCCCCCAAGGACA      360
H                   CCCAGGTCAGCAAGGGCACCTGCGAGCAAGGTCCTTCCATCGTGACACCCCCCAAGGACA      360
RR                  CCCAGGTCAGCAAGGGCACCTGCGAGCAAGGTCCTTCCATCGTGACACCCCCCAAGGACA      360
                    ************************************************************

XM_025290001.3      TCTGGAATGTCACTGGTGCC        380
H                   TCTGGAATGTCACTGGTGCC        380
RR                  TCTGGAATGTCACTGGTGCC        380
                    *******************
```

Figure S12. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_025290001.3| and *IGFBP7* marker (380-bp) sequences.

```
AF387814.1      CAGTCCGTCTCCTCCAAACAGAGGGTCACTGGTTTGGACTTCATCCCTGGGCTCCACCCT 60
H               CAGTCCGTCTCCTCCAAACAGAGGGTCACTGGTTTGGACTTCATCCCTGGGCTCCACCCT 60
RR              CAGTCCGTCTCCTCCAAACAGAGGGTCACTGGTTTGGACTTCATCCCTGGGCTCCACCCT 60
                ************************************************************

AF387814.1      CTCCTGAGTTTGTCCAAGATGGACCAGACATTGGCGATCTACCAACAGATCCTCACCAGT 120
H               CTCCTGAGTTTGTCCAAGATGGACCAGACATTAGCGATCTACCAACAGATCCTCACCAGT 120
RR              CTCCTGAGTTTGTCCAAGATGGACCAGACATTGGCGATCTACCAACAGATCCTCACCAGT 120
                ****************************** ************************** 

AF387814.1      CTGCCTTCCAGAAATGTGGTCCAAATATCTAATGACCTGGAGAACCTCCGGGACCTTCTC 180
H               CTGCCTTCCAGAAATGTGGTCCAAATATCTAATGACCTGGAGAACCTCCGGGACCTTCTC 180
RR              CTGCCTTCCAGAAATGTGGTCCAAATATCTAATGACCTGGAGAACCTCCGGGACCTTCTC 180
                ************************************************************

AF387814.1      CACCTGCTGGCCGCCTCCAAGAGCTGCCCCTTGCCGCAGGTCAGGGCCCTGGAGAGTTTG 240
H               CACCTGCTGGCCGCCTCCAAGAGCTGCCCCTTGCCGCAGGTCAGGGCCCTGGAGAGTTTG 240
RR              CACCTGCTGGCCGCCTCCAAGAGCTGCCCCTTGCCACAGGTCAGGGCCCTGGAGAGTTTG 240
                ********************************** ************************

AF387814.1      GAGAGCTTGGGCGTCGTCCTGGAAGCCTCCCTCTACTCCACCGAGGTGGTGGCCCTGAGC 300
H               GAGAGCTTGGGCGTCGTCCTGGAAGCCTCCCTCTACTCCACCGAGGTGGTGGCCCTGAGC 300
RR              GAGAGCTTGGGCGTCGTCCTGGAAGCCTCCCTCTACTCCACCGAGGTGGTGGCCCTGAGC 300
                ************************************************************

AF387814.1      CGGCTGCAGGGGTCACTACAGGACATGTTGCGGCAGCTGGACCTCAGTCCTGGGTGCTGA 360
H               CGGCTGCAGGGGTCACTACAGGACATGTTGCGGCAGCTGGACCTCAGTCCTGGGTGCTGA 360
RR              CGGCTGCAGGGGTCACTACAGGACATGTTGCGGCAGCTGGACCTCAGTCCTGGGTGCTGA 360
                ************************************************************
```

Figure S13. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|AF387814.1| and *LEP* marker (360-bp) sequences.

*MTNR1A, TSHR, CSGALNACT1, CADM2, ZNF503, KRR1, MTPN, IGFBP7, LEP,*and *ABCC4*) genes in repeat breeder and healthy Italian buffaloes were identified in this study utilizing sequenced amplified PCR products.The findings show that there are differences between the SNPs involving the two categories. The assessed buffaloes had a considerable nucleotide polymorphism dispersion ($p < 0.01$), according to the Fisher's exact test. It is crucial to emphasize that, when compared to the relevant datasets obtained from GenBank, the variations discovered and materials readily accessible in this context offer fresh information on the markers under consideration.

```
XM_045162483.1      GCAGAAGGCGAACTTCTGCTCGCGCCTGTTTGTCTGGTGGCTAAACCCCTTGTTTAAAAT      60
H                   GCAGAAGGCGAACTTCTGCTCGCGCCTGTTTGTCTGGTGGCTAAACCCCTTGTTTAAAAT      60
RR                  GCAGAAGGCGAACTTCTGCTCGCGCCTGTTTGTCTGGTGGCTAAACCCCTTGTTTAAAAT      60
                    ************************************************************

XM_045162483.1      TGGTCACAAACGGAAATTAGAACCAGATGACATGTACTCGGTGCTTCCAGAAGATCGCTC      120
H                   TGGTCACAAACGGAAATTAGAACCAGATGACATGTACTCGGTGCTTCCAGAAGATCGCTC      120
RR                  TGGTTACAAACGGAAATTAGAACCAGATGACATGTACTCGGTGCTTCCGGAAGATCGCTC      120
                    **** ******************************************** ***********

XM_045162483.1      CCAGTGCCTTGGAGAAGAGCTGCAAGGGTACTGGGATCAAGAAGTTAAGAGAGCCCAGAA      180
H                   CCAGTGCCTTGGAGAAGAGCTGCAAGGGTACTGGGATCAAGAAGTTAAGAGAGCCCAGAA      180
RR                  CCAGTGCCTTGGAGAAGAGCTGCAAGGGTACTGGGATCAAGAAGTTAAGAGAGCCCAGAA      180
                    ************************************************************

XM_045162483.1      GGATGGACAGGAACCCTCTTTAGTGAAAGCAATCGTAAAGTGTTACTGGAAATCCTATTT      240
H                   GGATGGACAGGAACCCTCTTTAGTGAAAGCAATCGTAAAGTGTTACTGGAAATCCTATTT      240
RR                  GGATGGACAGGAACCCTCTTTAGTGAAAGCAATCGTAAAGTGTTACTGGAAATCCTATTT      240
                    ************************************************************

XM_045162483.1      GGATGGACAGGAACCCTCTTTAGTGAAAGCAATCGTAAAGTGTTACTGGAAATCCTATTT      300
H                   GGATGGACAGGAACCCTCTTTAGTGAAAGCAATCGTAAAGTGTTACTGGAAATCCTATTT      300
RR                  GGATGGACAGGAACCCTCTTTAGTGAAAGCAATCGTAAAGTGTTACTGGAAATTCTATTT      300
                    ************************************************** ******

XM_045162483.1      AATTTGGGGAATGTTTACATTTCTTGAGGAAGGCACCAGAGTAGTTCAACCCATA  355
H                   AATTTTGGGAATGTTTACATTTCTTGAGGAAGGCACCAGAGTAGTTCAACCCATA  355
RR                  AATTTGGGGAATGTTTACATTTCTTGAGGAAGGCACCAGAGTAGTTCAACCCATA  355
                    ***** ***************************************************
```

Figure S14. Analysis of nitrogenous bases matching for DNA in healthy and repeat breeder buffaloes using GenBank gb|XM_045162483.1| and *ABCC4* marker (355-bp) sequences.

In recent studies, unique genes that are specifically related to the prevalence of bovine repeat breeders have been discovered using genome-wide association analysis for our investigated markers (Li et al., 2018; Mahrous et al., 2022), however, no research have yet looked into the relationship between repeat breeder risk and the SNPs in these genes. The gene sequences from the *Bubalus bubalis* employed in our study, which was published in PubMed, are the first to show this connection. The variance of the reproductive (*MFSD14A, PALMD, VPS13B, BMP, MTNR1A, TSHR, CSGALNACT1, CADM2, ZNF503, KRR1, MTPN, IGFBP7, LEP,*and *ABCC4*) markers and how they relate to repeat breeder in Italian buffaloes have not, to our knowledge, been the subject of any prior research. However, the candidate gene technique has been used to monitor the validity of bovine fertility features. For instance, Egyptian buffaloes showed a connection between *IGF-I* genetic variations and issues with reproduction (Ramadan et al., 2018). Buffaloes with *MTNR1A* gene polymorphisms had seasonal suppression of fertility (Rangashamaiahet al., 2022).The frequency of repeat breeding in Egyptian buffaloes have been investigated in relation to the polymorphisms of the *LEP*, and *BMP* genes (Mahrous et al., 2022; El-Debaky et al.,2020). Buffalo genetic variations in the *FSHR* and *ESR* genes and were correlated with the frequency of repeat breeders (Fouda et al., 2021). *LHR* gene polymorphisms and Egyptian buffalo fertility was also investigated (Sosa et al., 2016). *CYP19A1* gene polymorphisms were associated with anoestrus susceptibility and inactive ovaries in water buffaloes when a candidate gene approach to anoestrus was investigated (El-Bayomi et al., 2018; Abbaset al.,2014). Also discovered in Murrah buffaloes were polymorphisms in the *HSP70* gene, which may be related to post-partum anoestrus (Kumar et al., 2017).

The primary source of adaptation and selection is mutation (Chu& Wei, 2019). All of the reproductive markers that were being studied in this situation had exonic region mutations, which led to altered coding DNA sequences in repeat breeder buffaloes compared to healthy ones. Using DNA sequencing of reproductive genes, 30 SNPs were found; 16 of them are synonymous, and 15 are non-synonymous. Non-synonymous mutations alter protein sequences, and people who have these mutations are frequently the subject of natural selection (Chu, & Wei, 2019). The encoded amino acid at the mutant location is altered by genetic variation brought on by non-synonymous SNPs, which can result in structural and functional alterations in the mutated protein (Dakal et al., 2017). For a very long time, it was believed that selection on synonymous mutations was either nonexistent or very weak (Chu& Wei, 2019). In order to accurately characterize the investigated reproductive genes at the molecular level and to understand the physiological differences in reproductive performance between normal and repeat breeder buffaloes, our study discovered polymorphisms on the basis

Table 2. Single base differential dissemination as well as category of inherited alteration for reproductive markers in normal and repeat breeder buffaloes.

Gene	SNPs	Normal *n* = 120	Repeat breeder *n* = 120	Total *n* = 240	kind of inherited alteration	Amino acid order and sort
MFSD14A	C165T	-	72	72/240	Synonymous	55 C
	T322C	-	85	85/240	Synonymous	108 G
PALMD	T108C	91	-	91/240	Synonymous	36 L
VPS13B	T175C	48	-	48/240	Non-synonymous	59 C to R
	T223C	-	63	63/240	Non-synonymous	75 Y to H
	T47C	-	98	98/240	Non-synonymous	16 V to A
BMP	C80A	58	-	58/240	Non-synonymous	27 P to Q
	G184A	76	-	76/240	Non-synonymous	62 V to I
MTNR1A	C72T	49	-	49/240	Synonymous	24 N
	C87T	-	83	83/240	Synonymous	29 S
TSHR	T126C	-	38	38/240	Synonymous	42 T
	C177T	69	-	69/240	Synonymous	59 I
	C372T	-	103	103/240	Synonymous	124 V
CSGALNACT1	G135A	47	-	47/240	Synonymous	45 T
	G150A	91	-	91/240	Synonymous	50 R
CADM2	T168C	63	-	63/240	Synonymous	56 N
	G354T	39	-	39/240	Synonymous	118 T
ZNF503	G217C	-	98	98/240	Non-synonymous	73 G to R
KRR1	G168A	107	-	107/240	Synonymous	56 A
MTPN	A45G	46	-	46/240	Synonymous	15 L
	T189C	-	104	104/240	Synonymous	63 A
	G38C	-	58	58/240	Non-synonymous	13 R to P
IGFBP7	T107C	-	112	112/240	Non-synonymous	36 V to A
	A161C	92	-	92/240	Non-synonymous	54 Q to P
LEP	G93A	71	-	71/240	Synonymous	31 L
	G216A	-	86	86/240	Synonymous	72 P
ABCC4	C65T	-	89	89/240	Non-synonymous	22 S to L
	A109G	-	109	109/240	Non-synonymous	37 R to G
	C294T	-	74	74/240	Synonymous	98 I
	G306T	93	-	93/240	Non-synonymous	102 L to F

Single base difference dispersal for reproductive markers in normal and repeat breeder buffaloes showed a highly significant variation ($p< 0.01$) according to Fisher's exact analysis.

MFSD14A= Major facilitator superfamily domain containing 14A; *PALMD*= Palmdelphin; *VPS13B*= Vacuolar protein sorting 13 homolog B; *BMP*= Bone morphogeneic protein; *MTNR1A*= Melatonin receptor 1B; *TSHR*= Thyroid stimulating hormone receptor; *CSGALNACT1*= Chondroitin sulfate N-acetylgalactosaminyltransferase 1; *CADM2*= Cell adhesion molecule 2; *ZNF503*= Zinc finger protein 503; *KRR1* = Small subunit processome component homolog; *MTPN*= Myotrophin; *IGFBP7*= insulin like growth factor binding protein 7; *LEP*= Leptin; and *ABCC4*= ATP-binding cassette sub-family C member 4. A = Alanine; C = Cisteine; F = Phenylalanine; G = Glycine; H = Histidine; I = Isoleucine; L = Leucine; N= Asparagine; P= Proline; Q= Glutamine; R = Argnine; S = Serine; T = Threonine; V = Valine; and Y= Tyrosine.

of translated DNA sequence to be of greater value than intronic parts.

Role of investigated genes in reproduction

A protein-coding gene called major facilitator superfamily domain containing 14A (MFSD14A) has product homology to the family of solute carrier proteins (Doran et al., 2016). Additionally, MFSD14A functions as a component of cellular fundamentals, a molecular controller of transporter action, and a biological tool involved in trans-membrane transport (Sreedharan et al., 2011). The nuclear transfer transcripts in pig blastocyst stage were discovered to be negatively controlled by *MFSD14A* (Whitworth, et al.,2011), and the gene may also play a role in mouse spermatogenesis (Doran, 2016). Two SNPs in the *MFSD14A* gene have been linked to characteristics of buffalo milk production (Liu et al., 2018). A cytosolic protein involved in the phosphorylation of p53 is encoded by the palmdelphin (*PALMD*) (Moioli et al., 2013). The variance in milk yield is likely to be caused by the *PALMD* gene (Seo et al., 2016; Moioli, et al., 2013). *PALMD* polymorphisms had a significant impact on back fat thickness in a research to identify probable causative mutations associated to pig production attributes (Martínez-Montes et al., 2017). These *PALMD* functional studies suggest that it may be a key target gene that regulates variables related to reproductive performance.

The eye, hematological system, and central nervous system all develop and function as a result of the potential trans-membrane protein that the vacuolar protein sorting 13 homolog B (*VPS13B*) gene encodes (Liu et al., 2018). Protein transport has been identified as the primary biological utility of VPS13B, and it may play a role in vesicle-mediated transport as well as protein sorting within cells. In a prior investigation, *VPS13B* was discovered inside a QTL linked to the shape of the legs in dairy cattle (Van den Berg et al., 2014). Additionally, it was hypothesized that attributes related to milk production in Holstein cattle as well as female fertility were linked to the genomic region on BTA14 encompassing *VPS13B* (Capitan et al., 2014).

The secreted factor known as transforming growth factor (TGF) was initially discovered to influence cell proliferation (Roberts & Sporn, 1990). The proteins can be divided into two main groups according to cell signaling pathways: activins/transforming growth factors and bone morphogenetic proteins/growth differentiation factors (Chang et al., 2002). Among the BMP family memberships expressed inside the uterus, ovary, granulosa cells, and oocyte are BMP 2, 3, 4, 5, 6, 7 and 15 (Tanwar& McFarlane, 2011; Sun et al., 2010; Otsukaet al., 2001). They provide significant biological tasks as controllers of ovarian follicle growth, female reproductive tract discrepancy, blastocyst embedding in the uterus, and organogenesis and morphogenesis during embryo expansion (Kishigami & Mishina, 2005). The *BMP* gene has been the subject of numerous investigations in mammals, including cattle (Balozaet al., 2017), sheep (Ibrahim, 2019), and goats (Latifah & Saa, 2021; Ortiz et al., 2015). The blastocyst rate was shown to be substantially correlated with *BMP* SNPs by Lari et al., (2012).

Photoperiod and melatonin have been identified as the hypothalamus level modulators of the reproductive axis in a number of ovine investigations (Karsch et al., 2013). The pineal gland produces melatonin hormone in direct proportion to the length of darkness (Malpaux et al., 2001). The nighttime plasma melatonin content was highest in winter and lowest in summer in the Mediterranean buffaloes that had a seasonal reproductive tendency (Parmeggianiet al., 1994). Subcutaneous insertion of sustained-release melatonin implants in buffaloes during summer anestrus lengthens the daily presence of melatonin, simulating the effect of short days, and has been extensively investigated for the activation of the reproductive axis (Kavitaet al., 2018). Only the melatonin receptor 1A gene (MTNR1A) is connected to reproductive seasonality of the G-protein couple receptors melatonin receptor 1A gene (*MTNR1A*) and melatonin receptor 1B gene (MTNR-RIB), to which melatonin binds (Dubocovich et al., 2020).

Animal reproduction research have used hormones and antibiotics as therapies because hormones are crucial to animal reproduction (Refsdal, 2000). Seasonal cycles of body weight and reproduction are significantly influenced by thyroid hormones (Barrett et al., 2007). By chance, buffalo are a species that go into estrus seasonally. Thyroid hormone resistance (THR) has also been shown to be highly correlated with the THR mutation (Işıket al., 2013). The genes involved in the metabolic pathways that impact animal fertility have already been extensively studied (Morfeld & Brown, 2014). One of these genes, *CSGALNACT1*, is necessary for healthy cartilage development (Watanabe et al., 2010). The *CSGALNACT1* gene was hypothesized to affect bovine follicle growth by altering the degree of expression that controls glucose metabolism (Li et al., 2018). Buffalo reproductive efficiency was discovered to be

correlated with cell adhesion molecule 2 (CADM2) (Song et al., 2013). One SNP at the bovine *CADM2* gene was connected to the number of days between conception and the first calving as well as the first and second calving (Li et al.,2018).

The target genes for buffalo reproductive features include *ZNF503*, *KRR1*, and *MTPN* (Lappalainen et al., 2013). ZNF503 enhances cell invasion and proliferation in mammary epithelial cells, which has a key role in embryogenesis (Shahi et al., 2015; Chang et al.,2013). KRR1 has been linked to polycystic ovarian syndrome (PCOS) in European populations (Day et al.,2015). MTPN is crucial for the development of cells and skeletal muscles (Makinaet al., 2015) and is associated with the process of antigen recognition, a crucial step in the immune reaction (Wang & Wang, 2012). The elevated expression of these three genes in buffalo granulosa cells raises the possibility that they took part in dominant follicle selection (Li et al., 2018). IGFBP7 and follistatin share sequence similarity (Kato, 2000), the latter of which was once thought to be an inhibitor of FSH secretion (Jorgezet al., 2004) and is essential for ovarian and folliculogenesis (Muttukrishna et al., 2004). Additionally, *IGFBP7* was discovered to be expressed in the granulosa cells of the large antral follicles of the pig ovary (Wandji et al., 2002) and the bovine corpus luteum (Casey et al., 2004). Therefore, we assume that IGFBP7 is in some way responsible for the regulation of follicle development and ovulation (Li et al., 2018).

The versatile peptide hormone leptin, which is mostly generated by adipocytes, plays a crucial role in reproduction alongside controlling energy expenditure and body weight (Wiles et al., 2014). Leptin and its receptors have been discovered to be expressed in buffalo ovaries. Contrary to the widely held idea that leptin primarily affects the neuroendocrine component of reproduction, this clearly demonstrates that leptin directly engages in ovarian activity (Reshma et al., 2016). According to Fu et al., (2016), the ovary has leptin receptors that can control steroidogenesis and improve the oocyte's ability to support later embryonic development. The *ABCC4* gene was linked to the number of services per conception (NSC). Since it affects prostaglandin efflux from cells, this gene appears to be crucial to support pregnancy in the endometrium of pregnant cows(Spencer etal., 2013) and pigs (Seo et al., 2014). Prostaglandin plays a number of roles in reproduction, including conceptus implantation and maternal recognition of pregnancy—processes that are intimately connected to NSC. The expression of *ABCC4* was substantially linked with residual feed intake (RFI) in Angus cattle and was up-regulated in animals with high RFI (Chenetal., 2012). Nucleotide sequence variants in *ABCC4*was linked to marbling score in Nelore cattle (Feitosa et al., 2014). The recently proposed theory is that *ABCC4*, which is highly polymorphic and acts in fundamental metabolic pathways, may have effects on reproductive and meat quality (De Camargo et al., 2015).

CONCLUSIONS

Using PCR-DNA sequencing, single nucleotide variations (SNPs) in the genes were found for reproductive (*MFSD14A, PALMD, VPS13B, BMP, MTNR1A, TSHR, CSGALNACT1, CADM2, ZNF503, KRR1, MTPN, IGFBP7, LEP,*and *ABCC4*) indicators in normal and repeat breeder Italian buffaloes. These special functional variations present a promising opportunity to lower the occurrence of repeat breeders by using genetic markers in conjunction with normal wellbeing during buffalo selection. Future approaches to dealing with repeat breeders may be facilitated based on the gene domains here.

FUNDING

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R318), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, funds the publishing of this study. This research was also funded by the Researchers Supporting Project number (RSP-2024/R232), King Saud University, Riyadh, Saudi Arabia.

ACKNOWLEDGMENTS

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R318), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia acknowledged by the authors. The authors sincerely acknowledge the Researchers Supporting Project for funding this work number (RSP-2024/R232) at King Saud University, Riyadh, Saudi Arabia.

CONFLICT OF INTEREST

There are no competing interests, according to the authors.

REFERENCES

Abbas, H. S., El-Magd, M. A., El-Bayomi, K. M. & Sosa, G. A. (2014). Detection of SNPs in exon7 locus of Cyp19 gene and their association with anestrus in Egyptian buffaloes (Bubalus bubalis). *Benha Veterinary Medical Journal,* 26, 151–160.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biol*ogy, 215, 403–410.

Ashwell, M.S., Rexroad Jr, C. E., Miller, R. H., Van Raden, P.M. & Da, Y. (1997). Detection of loci affecting milk production and health traits in an elite US Holstein population using microsatellite markers. *Animal Genetics*, 28, 216-222.

Baloza, S. H., Abo-Salem, M. E. S. & Hemeda, S. A. (2017). Effect of genetic variations in BMP 4 gene (exon 2 plus part of intron 2) on infertility in Egyptian Buffalo cows.*Benha Journal of Applied Sciences,* 2, 37–41.

Barrett, P., Ebling, F. J. P., Schuhler, S., Wilson, D., Ross, A.W., Warner, A, Jethwa, P., Boelen, A., Visser, T. J., Ozanne, D. M. (2007). Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology*, 148, 3608–17.

Berry, D.P. & Evans, R. (2014). Genetics of reproductive performance in seasonal calving beef cows and its association with performance traits. *Journal of Animal Sci*ence, 92, 1412–1422.

Bishop, M. D., Hawkins, G.A. & Keeler, C.L. (1995). Use of DNA markers in animal selection. *Theriogenology,* 43, 61-70.

Boesenberg-Smith, K. A., Pessarakli, M. M. & Wolk, D. M. (2012). Assessment of DNA Yield and Purity: An Overlooked Detail of PCR Troubleshooting. *Clinical Microbiology Newsletter,* 34, 1–6.

Boichard, D. (1990). Estimation of the economic value of conception rate in dairy cattle.*Livestock Production Science Journal*, 24, 187–204.

Boom, R., Sol, C. J., Salimans, M. M. & Jansen, C. L. (1990). Wertheim-van Dillen PM, Noordaa JVD. Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology,* 28, 495–503.

Buch, L.H., Kargo, M., Berg, P., Lassen, J. & Sørensen A. C. (2012). The value of cows in reference populations for genomic selection of new functional traits. *Animals*, 6, 880–886.

Cammack, K., Thomas, M. & Enns, R. (2009). Reproductive traits and their heritabilities in beef cattle. *The Professional Animal Scientist Journal*, 25, 517–528.

Capitan, A., Michot, P., Baur, A., Saintilan, R., Hoze, C., Valour, D., Guillaume, F., Boichon, D., Barbat, A. &Boichard, D. (2014). Genetic tools to improve reproduction traits in dairy cattle. *Reproduction Fertility Development,* 27, 14–21.

Casey, O. M., Fitzpatrick, R., Mcinerney, J. O., Morris, D. G., Powell, R., &Sreenan, J. M. (2004). Analysis of gene expression in the bovine corpus luteum through generation and characterisation of 960 ESTs.*Biochimica et Biophysica Acta - Gene Structure and Expression,* 1679, 10–7

Chang, H., Brown, C.B. &Matzuk, M.M. (2002). Genetic analysis of the Mammalian transforming growth factor superfamily. *Endocrinology Reviews,* 23,787–823.

Chang, S. L., Liu, Y. C., Chen, S. Y., Huang, T. H., Liu, P. T. & Liu, F. C. (2013). Identification of two evolutionarily conserved 5′ cis-elements involved in regulating spatiotemporal expression of Nolz-1 during mouse embryogenesis. *PLOS One*, 8, e54485.

Chen, Y., Arthur, P. F., Barchia, I. M., Quinn, K., Parnell, P. F., Herd, R. M. (2012). Using gene expression information obtained by quantitative real-time PCR to evaluate Angus bulls divergently selected for feed efficiency. *Animal Production Science*, 52, 1058–67.

Chu, D. & Wei, L. (2019). Nonsynonymous, synonymous and nonsense mutations in human cancer-related genes undergo stronger purifying selections than expectation. *BMC Cancer*, 19, 359.

Dakal, T.C., Kala, D., Dhiman, G., Yadav, V., Krokhotin, A. &Dokholyan, N.V. (2017). Predicting the functional consequences of non-synonymous single nucleotide polymorphisms in IL8 gene. *Scientific Reports,* 7, 6525.

Das, G.K. & Khan, F.A. (2010). Summer anestrus in buffalo-A review. *Reproduction in Domestic Animals,*45:e483-e494.

Day, F. R., Hinds, D. A., Tung, J. Y., Stolk, L., Styrkarsdottir, U., Saxena, R., Bjonnes, A., Broer, L., Dunger, D. B. & Halldorsson, B. V. et al. (2015). Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nature Commun*ication, 6, 8464.

De Camargo, G. M., Aspilcueta-Borquis, R. R., Fortes, M. R., Porto-Neto, R., Cardoso, D. F., Santos, D. J., Lehnert, S. A., Reverter, A., Moore, S. S. &Tonhati, H. (2015). Prospecting major genes in dairy buffaloes. *BMC Genomics*, 16, 872.

Dekkers, J. (1991). Estimation of economic values for dairy cattle breeding goals: bias due to sub-optimal management policies. *Livestock Production Science Journal,* 29, 131–149.

Devkota, B., Shah, S. & Gautam, G. (2022). Reproduction and Fertility of Buffaloes in Nepal. *Animals.* 13, 70.

Doran, J., Walters, C., Kyle, V., Wooding, P., Hammett-Burke, R. &Colledge, W.H. (2016). Mfsd14a (Hiat1) gene disruption causes globozoospermia and infertility in male mice. *Reproduction,* 152, 91– 99.

Dubocovich, M. L., Cardinali, D. P., Delagrange, P. R. M., Krause, D. N., Strosberg,D. &. Sugden, D. (2000). Melatonin Receptors. In Girdlestone, D. (ed.) The IUPHAR Compendium of Receptor Characterization and Classifcation, 2nd ed. London, UK. 270-277.

El-Bayomi, K. M., Saleh, A. A., Awad, A., El-Tarabany, M. S., El-Qaliouby, H. S., Afifi, M., El-Komy, S., Essawi, W. M., Almadaly, E.A. & El-Magd, M. A. (2018). Association of CYP19A1 gene polymorphisms with anoestrus in water buffaloes. *Reproduction and Fertility Development*, 30, 487-497.

El-Debaky, H., Mahmoud, K. M., Abd El-Razik, K. A., Sosa, A. S. A., Kandiel, M. M. M. & Ahmed, Y. F. (2020). PCR-SSCP and Sequencing Analysis for Studying Leptin Gene Polymorphism and Its Association with Reproductive Status of Egyptian Buffalo. *Egyptian Journal of Veterinary Science,* 51, 11-21.

Feitosa, F. L. B., Pereira, A. S. C., Venturini, G. C., Tonussi, R. L., Espigolan, R., & Gordo, D. M. et al. (2014). Genome wide association study between copy number variation regions with marbling score in Nelore cattle. In: Proceedings, 10th World Congress of Genetics Applied to Livestock Production. Vancouver.

Fouda, M., Hemeda, S., El-Bayomi, K., El-Araby, I., Hendam, B. & Ateya, A. (2021). Genetic polymorphisms in FSHR/ALUI and ESRα /BG1I loci and their association with repeat breeder incidence in buffalo. *Journal of the Hellenic Veterinary Medical Society,* 72, 2869-2878.

Fu, Y.F., Li, L., Li, B., Fang, X. & Ren, S. (2016). Long form leptin receptor and SNP effect on reproductive traits during embryo attachment in Suzhong sows. *Animal Reproduction Science,* 168, 57–65.

Heidari, M., Kafi, M., Mirzaei, A., Asaadi, A. & Mokhtari, A. (2019). Effects of follicular fluid of preovulatory follicles of repeat breeder dairy cows with subclinical endometritis on oocyte developmental competence. *Animal Reproduction Science,* 205, 62–69.

Ibrahim, A. H. M. (2019). Association of growth performance and body conformational traits with BMP4 gene variation in Barki lambs. *Growth Factors*, 37, 153–163.

Işık, E., Beck, P. P., Campi, I., Özon, A., Alikaşifoğlu, A., Gönç, N. & Kandemir N. (2013). Thyroid hormone resistance: a novel mutation in thyroid hormone receptor beta (THRB) gene - case report. *Turkish Journal of Pediatrics.* 55, 322–7

Jorgez, C. J., Klysik, M., Jamin, S. P., Behringer, R. R., &Matzuk, M. M. (2004). Granulosa cell specific inactivation of follistatin causes female fertility defects. *Molecular Endocrinol*ogy, 18, 953–67.

Karsch, F. J., Bittman, E. L., Foster, D. L., Goodman, R. L., Legan, S. J. & Robinson, J. E. (2013). Neuroendocrine basis of seasonal reproduction. Recent Progress in Hormone Research, Academic Press, New York, USA.

Kato, M. V. (2000). A secreted tumor-suppressor, mac25, with activin-binding activity. *Molecular Medicine,* 6, 126–35

Kavita, K., Phogat, J. B., Pandey, A. K., Balhara, A. K., Ghuman, S. S. & Gunwant, P. (2018). Effects of melatonin supplementation prior to Ovsynch protocol on ovarian activity and conception rates in anestrous Murrah bufalo heifers during out of breeding season. *Reproductive Biology,* 18, 161-168.

Kishigami, S. & Mishina, Y. (2005). BMP signaling and early embryonic patterning. *Cytokine Growth Factor Reviews,* 16, 265– 278.

Kukurba, K. R. & Montgomery, S. B. (2015). RNA Sequencing and Analysis. *Cold Spring Harbor Protocols*, 13, 951-69.

Kumar, R., Ghosh, M., Kumar, N., Balhara, A. K., Gupta, M., Sharma, R. K. & Singh, I. (2017). Polymorphism in 5' untranslated region of heat-shock protein 70 gene as marker of post-partum anoestrus in Murrah buffaloes. *Reproduction Domestic Animal*, 52, 505-512.

Lappalainen, T., Sammeth, M., Friedlander, M. R., Hoen, P. A, Monlong, J., Rivas, M. A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T. & Ferreira, P. G. et al. (2013). Transcriptome and genome sequencing uncovers functional variation in humans. *Nature*, 501, 506–11.

Lappalainen, T., Sammeth, M., Friedlander, M. R., Hoen, P. A., Monlong, J., Rivas, M. A., Gonzalez-Porta, M., Kurbatova, N., Griebel, T. & Ferreira, P. G. et al. (2013). Transcriptome and genome sequencing uncovers functional variation in humans. *Nature*, 501, 506–11.

Lari, A. M., Mohebbi-Fani, M. & Rowshan-Ghasrodashti, A. (2012). Causes of culling in dairy cows and its relation to age at culling and interval from calving in Shiraz, Southern Iran. *Veterinary Research Forum*, 3, 233–237.

Latifah, L. & Saa N.R. (2021). Single nucleotide Polymorphism gen BMP4 pada kambingberdasarkan data GenBank. *Journal of Tropical Animal Research,* 2,1, 24–29

Li, J., Liu, J., Campanile, G., Plastow, G., Zhang, C., Wang, Z., Cassandro, M., Gasparrini, B., Salzano, A., Hua, G., Liang, A. & Yang, L. (2018). Novel insights into the genetic basis of buffalo reproductive performance. *BMC Genomics*, 9, 814.

Li, J., Liu, X., Yang, J., Wang, H., Jiang, J., Liu, L., He, S., Ding, X., Liu, J. & Zhang, Q. (2014). Targeted resequencing of GWAS loci reveals novel genetic variants for milk production traits. *BMC Genomics*, 15, 1–9.

Liu, J. J., Liang, A. X., Campanile, G., Plastow, G., Zhang, C., Wang, Z., Salzano, A., Gasparrini, B., Cassandro., M. & Yang, L. G. (2018). Genome-wide association studies to identify quantitative trait loci affecting milk production traits in water buffalo. *Journal of Dairy Science,* 101, 433-444.

Mahrous, K. F., El-Kader, H. A. M., Abdelhafez, M. A., Aboelenin, M. M., Balabel, E. A., Mabrouk, D. M., El Malky, O. M. &Hassanan, M. S. (2022). Genetic structure of some candidate genes of repeat breeder syndrome in Egyptian buffaloes. *Journal of genetic engineering and biotechnology,* 20, 110.

Makina, S. O., Muchadeyi, F. C., Van Marle-Koster, E., Taylor, J. F., Makgahlela, M. L., &Maiwashe, A. (2015). Genome-wide scan for selection signatures in six cattle breeds in South Africa. *Genetics Selection Evolution,* 47, 92.

Malpaux, B., Migaud, M., Tricoire, H. &Chemineau, P. (2001). Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *Journal of Biological Rhythms.* 16, 336-347.

Martínez-Montes, A. M., Fernandez, A., Pérez-Montarelo, D., Alves, D., Benitez, R., Nunez, Y., Ovilo, C., Ibañez-Escriche, N., Folch, J. & Fernandez, A. (2017). Using RNA-Seq SNP data to reveal potential causal mutations related to pig production traits and RNA editing. *Animal Genetics,* 48, 151–165.

Moioli, B., Scatà, M. C., Steri, R., Napolitano, F. & Catillo, G. (2013). Signatures of selection identify loci associated with milk yield in sheep. *BMC Genetics*, 14, 76.

Moioli, B., Scatà, M., Catillo, G., Napolitano, F. & Pilla, F. (2013). Whole-genome genotyping for detecting mutations in the genes that are directly responsible for the trait variation. Page P0621 in Proc. Plant and Animal Genome XXI, San Diego, CA.

Morfeld, K. A. & Brown, J. L. (2014). Ovarian acyclicity in zoo African elephants (Loxodonta africana) is associated with high body condition scores and elevated serum insulin and leptin. *Reproduction, Fertility and Development,* 28, 640–7.

Musunuru, K., Strong, A., Frank-Kamenetsky, M., Lee, N. E., Ahfeldt, T., Sachs, K.V., Li, X., Li, H., Kuperwasser, N,& Ruda, V. M. (2010). From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. *Nature*, 466, 714–9.

Muttukrishna, S., Tannetta, D., Groome, N., & Sargent I. (2004). Activin and follistatin in female reproduction. *Molecular and Cellular Endocrinol*ogy, 225, 45–56.

Ortiz, W. H., Quirino, C. R., Silva, A., Oliveira, C. S., Serapiao, R. V., Pacheco, A. &Bartholazzi, A. (2015). Association between BMP4 gene polymorphism and in vitro embryo production traits in Gyr cows.*Revista Colombiana de CienciasPecuarias*, 28, 156–164.

Otsuka, F., Yamamoto, S., Erickson, G. F. & Shimasaki, S. (2001). Bonemorphogenetic protein-15 inhibits follicle stimulating hormone (FSH) action by suppressing FSH receptor expression. *Journal of Biological Chemistry,* 276, 11387-11392

Pal, A. & Chakravarty, A. K. (2020). Disease resistance for different livestock species. Genetics and Breeding for Disease Resistance of Livestock, 19, 271–96.

Parmeggiani, A., Di Palo, R., Zicarelli, L., Campanille, G., Esposito, L., Seren, E., Accorsi, P. A &Sofai, S. M. (1994). Melatonin and reproductive seasonality in the bufalo cow. *Agricoltura Ricerca*, 153, 41-48

Ramadan, S. I., Abo-Gamil, Z. H. & El-Qaliouby, H. S. (2018). Polymorphism of IGF-1 and Its Receptor (IGF-1R) Genes and Their Association with Fertility Problems of Egyptian Buffaloes. *Alexandria Journal of Veterinary Sciences,* 59, 119-124.

Rangashamaiah, M. M., Ghuman, S. S. & Cheema, R. (2022). Association of melatonin receptor 1a (MTNR1A) gene polymorphism with seasonal suppression of fertility in buffaloes. *Buffalo Bulletin*, 41, 749-763.

Refsdal, A.O. (2000). To treat or not to treat: a proper use of hormones and antibiotics. *Animal Reproduction Science,* 60, 109–19.

Regmi, G.& Dhakal, I.P. (2020). Systemic levels of iron, phosphorus, and total protein in normocyclic versus repeat breeder Holstein Friesian crossbred cows of Kesharbag, Chitwan. *Nepalese Veterinary Journal,* 13, 2353-2357.

Reshma, R., Mishra, S. R., Thakur, N., Parmar, M. S., Somal, A., Bharti, M. K., Pandey, S., Chandr, V., Chouhan, V. S., Verma, M. R., Singh, G., Sharm, G. T., Maurya, V. P.&Sarkara,M. (2016). Modulatory role of leptin on ovarian functions in water bufalo (Bubalus bubalis). *Theriogenology*, 86, 1720–1739.

Roberts, A.B. & Sporn, M. B. (1990). The transforming growth factors. In: Sporn, M.B., Roberts, A.B. (Eds.), Peptide Growth Factors and Their Receptors, Part I. *Springer-Verlag Berlin,* 419–472.

Roxström, A. & Strandberg, E. (2002). Genetic analysis of functional, fertility-, mastitis-, and production-determined length of productive life in Swedish dairy cattle.*Livestock Production Science Journal*, 125–135.

Sammad, A., Khan, M. Z., Abbas, Z., Hu, L., Ullah, Q., Wang, Y., Zhu, H. & Wang, Y. (2022). Major Nutritional Metabolic Alterations Influencing the Reproductive System of Postpartum Dairy Cows. *Metabolites*, 12, 60.

Sanger, F., Nicklen, S. & Coulson, A. R. (1997). DNA sequencing with chain-terminating inhibitors. *The Proceedings of the National Academy of Sciences*, USA, 74, 5463–5467.

Schaeffer, L. R. (2006). Strategy for applying genome-wide selection in dairy cattle. *Journal of Animal Breeding and Genetics*, 123, 218–223.

Seo, H., Choi, Y., Shim, J., Yoo, I., & Ka, H. (2014). Prostaglandin Transporters ABCC4 and SLCO2A1 in the Uterine Endometrium and Conceptus During Pregnancy in Pigs. *Biology of Reproduction,* 90, 5.

Seo, M., Lee, H. J., Kim, K., Caetano-Anolles, K., Jeong, J.Y., Park, S., Oh, Y. K., Cho, S. & Kim, H. (2016). Characterizing milk production related genes in Holstein using RNA-seq. Asian-australas. *Journal of Animal Science,* 29, 343.

Shahi, P., Slorach E.M., Wang, C.Y., Chou, J., Lu, A., Ruderisch, A. & Werb, Z. (2015). The transcriptional repressor ZNF503/Zeppo2 promotes mammary epithelial cell proliferation and enhances cell invasion. *Journal Biological Chemistry,* 290, 3803–13.

Shao, B., Sun, H., Ahmad, M. J., Ghanem, N., Abdel-Shafy, H., Du, C., Deng, T., Mansoor, S., Zhou, Y., Yang, Y., Zhang, S., Yang, L. & Hua, G. (2021). Genetic features of reproductive traits in bovine and Buffalo: lessons from bovine to Buffalo. *Frontiers in Genetics*,12, 617128.

Song, L. J., Wu, J. F., Yang, Z. B., Guo, L., Zhang S. J. & Wu, J.J. (2013). Investigation of transferability of BovineSNP50 BeadChip from cattle to water buffalo for genome wide association study. *Molecular Biology Reports,* 40, 743–50.

Sosa, A. S. A, Mahmoud, K. M., Kandiel, M. M. M., Eldebaky, H. A. A., Nawito, M. F. & Abou El Roos, M. E. A. (2016). Genetic Polymorphism of Luteinizing Hormone Receptor Gene in Relation to Fertility of Egyptian Buffalo.*Biotechnology Journal*, 12, 1-11.

Spencer, T. E., Forde, N., Dorniak, P., Hansen, T. R., Romero, J. J. & Lonergan, P. (2013). Conceptus-derived prostaglandins regulate gene expression in the endometrium prior to pregnancy recognition in ruminants. *Reproduction,* 146, 377–87.

Sreedharan, S., Stephansson, O., Schiöth, H. B. & Fredriksson, R. (2011). Long evolutionary conservation and considerable tissue specificity of several atypical solute carrier transporters. *Gene*, 478, 11–18.

Sun, R. Z., Lei, L., Cheng, L., Jin, Z. F., Zu, S. J. & Shan, Z. Y. (2010). Expression of GDF-9, BMP-15 and their receptors in mammalian ovary follicles. *Journal of Molecular Histol*ogy, 41, 325–32.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology Evolution,* 24, 1596–1599.

Tanwar, P.S. & McFarlane, J.R. (2011). Dynamic expression of bone morphogenetic protein 4 in reproductive organs of female mice. *Reproduction*, 142, 573–9.

Van den Berg, I., Fritz, S., Rodriguez, S., Rocha, D., Boussaha, M., Lund, M. S. &Boichard, D. (2014). Concordance analysis for QTL detection in dairy cattle: A case study of leg morphology. *Genetics Selection Evolution,* 46, 31.

Wandji, S. A., Gadsby, J. E, Barber, J. A, & Hammond, J. M. (2000). Messenger ribonucleic acids for IGFBP-7 and connective tissue growth factor (CTGF) are inversely regulated during folliculogenesis and early luteogenesis. *Journal of Endocrinology,* 141, 2648–57.

Wang, L. & Wang, Y. (2012). Molecular characterization, expression patterns and subcellular localization of Myotrophin (MTPN) gene in porcine skeletal muscle. *Molecular Biology Reproduction*, 39, 2733–8.

Warriach, H. M., Mcgill, D.M., Bush, R.D., Wynn, P.C. & Chohan, K.R. (2015). A review of recent developments in buffalo reproduction - a review. *Asian-Australasian Journal of Animal Sciences,* 28, 451–455.

Watanabe, Y., Takeuchi, K., Onaga, S. H., Sato, M., Tsujita, M., Abe, M., Natsume, R., Li, M., Furuichi, T. & Saeki, M. (2010). Chondroitin sulfate N-acetylgalactosaminyltransferase-1 is required for normal cartilage development. *Biochemistry Journal,* 432, 47–55.

Whitworth, K. M., Zhao, J., Spate, L. D., Li, R. & Prather, R. S. (2011). Scriptaid corrects gene expression of a few aberrantly reprogrammed transcripts in nuclear transfer pig blastocyst stage embryos. *Cellular Reprogramming,* 13, 191–204.

Wiles, J.R., Katchko, R.A., Benavides, E.A., O'Gorman, C.W., Escudero, J.M., Keisler, D.H., Stanko, R.L., & Garcia, M.R. (2014). The effect of leptin on luteal angio- genic factors during the luteal phase of the estrous cycle in goats. *Animal Reproduction Science,* 148, 121–129.

Yusuf, M., Toshihiko, N., Ranasinghe, B., Gautam, G., Long, S., Yoshida, C., Koike, K. & Hayashi, A. (2010). Reproductive performance of repeat breeders in dairy herds. *Theriogenology*, 73, 1220-1229.