Global Veterinaria 15 (2): 163-168, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.15.02.96166

Genotyping of Follicle Stimulating Hormone Receptor Gene in Fertile and Infertile Buffalo

[1]A.S.A. Sosa, [1]Karima Gh. M. Mahmoud, [1]H.A.A. Eldebaky,
[2]M.M.M. Kandiel, [2]M.E.A. Abou E l-Roos and [1]M. F. Nawito

[1]Department of Animal Reproduction & A.I., National Research Centre Giza, Egypt
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Kaliobia, Egypt

Abstract: This study aimed to estimate the effects of restriction fragment length polymorphism **[**RFLP] in the follicle stimulating hormone receptor gene [FSHR gene], regarding to fertile and infertile Egyptian buffaloes. The ovarian status of animals was classified by ultrasonography into follicular phase, luteal phase, bilateral inactive ovary with normal uterus and bilateral inactive ovary with endometritis. Blood samples were collected from fertile and non-fertile buffaloes with the history of anestrum or repeat breeders for DNA extraction and progesterone analysis. The results showed that, the overall mean of plasma progesterone levels in normal cyclic animals were significantly higher during the luteal phase (5.78±0.69 ng/ml) as compared to the follicular phase (1.57±0.18 ng/ml). Moreover, there were no significant differences between animals had normal uterus and those suffered from endometritis in association with bilateral inactive ovaries. All buffaloes investigated in this study were genotyped as CC where DNA amplified fragments at 306-bp were digested with *AluI endonuclease* and gave two digested fragments at 243- and 63-bp. In conclusion, monomorphic pattern of follicle-stimulating hormone receptor gene [FSHR gene] is considered a unique feature that may be related to the characteristic species in buffalo. So, the polymorphisms and interaction with the fertility feature should be endorsed for advanced research with a big number of buffaloes.

Key words: Buffalo · Fertility · FSHR · Infertility Nucleotide sequences · PCR-RFLP

INTRODUCTION

The productivity of buffaloes is considerably affected by inherent disorders such as low reproductive proficiency, which is mainly due to late maturity, poor expression of estrus, anestrus, inactive ovaries, prolonged postpartum interval, seasonal cyclicity and silent estrus [1]. Genes that impact the physiological and endocrine functions may regulate the inherent fertility and account for the genetic association of measures of early reproductive appropriateness and growth, milk and overall productivity [2]. The endocrine system is a major regulator of the reproductive functions through the hypothalamic–pituitary–gonad axis and its interactions. The FSH receptor gene is expressed in the gonads [3] and the actions of FSH are mediated to ovarian and testicular somatic cells through these receptors.

The FSH receptor gene is mapped to chromosome 2 p21 in the human. The human FSHR gene is about 215 kb in size and it comprises of 10 exons and 9 intervening introns [4]. The FSHR gene was studied in Bos Taurus, [5]. This gene is located on chromosome 11 and its structure is determined by 10 exons and 11 introns; the first 9 exons enclose the extracellular domain whereas exon 10 encloses the transmembrane domain [5].

Considering FSH importance in the maintenance of ovarian function, FSHR gene has been studied in many livestock species, including cattle [5], sheep [6], horse [7] and donkey [8]. In view of the lack of studies about the genetic polymorphisms linked to the reproductive characteristics in buffalo, the objective of the present study was to estimate the effects of RFLPs in the follicle stimulating hormone receptor gene regarding ovarian status and progesterone concentration in Egyptian buffaloes.

Corresponding Author: Karima Gh. M. Mahmoud, Department of Animal Reproduction & A.I., National Research Centre, Postal code: 12622 Giza, Egypt.
Tel: +2 0101827716; Fax: +2 02 33370931; E-mail: karima_mahmoud@hotmail.com.

MATERIALS AND METHODS

Animals: The present study was conducted on a total number of 150 Egyptian fertile and none-fertile buffaloes with the history of anestrum or repeat breeders from Qaluobia (Meet Kenana Village). The animals came to the veterinary clinic for treatment of infertility problems or confirmation of pregnancy in fertile animals. Ultrasonographic examination of all buffaloes was conducted through a transrectal ultrasonography with a B-mode scanner (Magic 2200, Eickemeyer Veterinary Equipment Inc., Germany). The ovarian status of animals was classified by ultrasonography into follicular phase, luteal phase, bilateral inactive ovary with normal uterus and bilateral inactive ovary with endometritis. Then blood samples were collected from all cases for progesterone evaluation and DNA extraction.

Progesterone Concentration: Blood samples were collected from the jugular vein of animals into non-heparinized Vacutainer tubes. Serum was harvested upon centrifugation at 3000 rpm for 20 min, labeled and stored at -20°C until assayed. Progesterone levels in sera were determined by the use of progesterone EIA kits (Cal Biotech Inc, California, USA) according to the method of Radwanska *et al.* [9].

DNA Extraction: Blood samples were collected into EDTA anticoagulant vacutainer tubes.

Genomic DNA was extracted from blood samples with the QIAamp DNA blood kit (QIAGEN GmbH, Hilden, Germany) as indicated by the manufacturer's directions.

PCR Reaction and DNA Amplification: The genetic polymorphism was analyzed using primers representing exon 10 in Egyptian buffalo FSHR gene. The primers used for amplification of FSHR gene fragments of 306-bp were those described by Lussier *et al.* [10], with the following nucleotide sequence:

Forward, 5\ CTGCCTCCCTCAAGGTGCCCCTC 3\
Reverse, 5\ AGTTCTTGGCTAAATGTCTTAGGGGG 3\

Amplification reactions were done in a final volume of 50 µL, containing 5µl buffer 10x, 1µ 2.5 mM (dNTPs mixture), 3µl 25 mM (MgCL2), 0.25µl primer, 0.3µl Taq polymerase (5U/µl), 35.2 µl water (nuclease free water), 5µl DNA sample. The reactions followed the sequence: one cycle at 95 °C for 4 min. (initial denaturation) and 30 cycles of the sequence: 95 °C for 60 sec., 55 °C for 30 sec. and 72 °C for 2 min. After the reaction was completed, products of PCR were exposed to electrophoresis in 2% agarose gel, TBE 1× buffer (1M Tris-HCl pH 7.4; 0.5 M EDTA pH8.0 and 10.8 g boric acid) with ethidium bromide, at 60 V for approximately 2 hrs. Visualization of the bands was done under ultraviolet Trans-illumination and a picture was taken in Gel-Doc System (Bio-Rad). The size of the amplified product was compared with the 100 bp Ladder DNA marker.

Restriction Fragment Length Polymorphism (RFLP) Technique: For genotyping, PCR product was digested with *AluI.* Gene fragments were subjected to digestion by restriction enzymes in a total volume of 20 µL (10 µL reaction solution, 2 µL enzyme buffers, 0.2 µL enzymes and 7.8 µL water) and placed in the thermocycler at 37 °C for 30 min. After digestion, the samples were quantified to visualize the amplified fragments by gel electrophoresis as mentioned in PCR with 3% agarose concentration.

Sequence Analysis: Before genetic sequencing, the PCR product was purified using QIAquick PCR purification kit (QIAGEN) and PCR purification spin protocol (QIAGEN), designed for the isolation of DNA fragments from PCR reactions. The PCR products were sequenced by Macrogen Incorporation (Seoul, South Korea). Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite.

Statistical Analysis: Mean ±SEM of progesterone concentration was tabulated and statistically computed by one way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) program Ver. 16.

RESULTS AND DISCUSSION

In the current work, Table (1) presented that the overall mean of plasma progesterone levels in normal cyclic animals were significantly higher during the luteal phase (5.78±0.69 ng/ml) as compared to that of the follicular phase (1.57±0.18 ng/ml). Moreover, there were no significant differences in progesterone levels between buffaloes with normal uterus and those suffered from endometritis associated with bilateral inactive ovaries. Progesterone levels rise and fall coincided with the growth and regression of the corpus luteum (CL) which is the principle source of progesterone in cycling buffalo [11]. Peripheral progesterone concentrations are minimal on the day of estrus (0.1 ng/ml), upsurge to

Table 1: Plasma progesterone level (ng/ml) in buffaloes with different ovarian phases

Ovarian status	Mean	±S.E.	Min.	Max.
Follicular phase	1.57[b]	0.18	0.50	3.10
Luteal phase	5.78[a]	0.69	3.10	11.60
Bilateral inactive ovary with normal uterus	1.95[b]	0.44	0.50	5.70
Bilateral inactive ovary with endometritis	1.87[b]	0.38	0.50	4.90

Values with different subscription with in the same column differ significantly ($P < 0.001$).

topmost concentrations of 1.6-3.6 ng/ml on days 13 to 15 of the cycle [11, 12] or even on day 17 [13] before declining to basal levels at the onset of the next estrus. Progesterone levels continue to increase in animals that conceive, but drop 3 days before the next estrus in those failed to conceive [14]. The onset of the decline in progesterone concentrations is inconstant, conditioned with the time of regression of CL. Mondal and Prakash [15] compared progesterone concentration in silent and estrous buffalo and found that progesterone levels augmented from 0.42±0.02 and 0.38±0.02 ng/ml during proestrus phase (Day -1 to 1, Day 0= day of estrus) to 0.66±0.12 and 0.51±0.07 ng/ml during early luteal phase (Day 2 to 5) and then further to 1.55±0.33 and 1.30±0.13 ng/ml during mid-luteal phase (Day 6 to 14) followed by its drop to 1.12± 0.27 and 0.66±0.13 ng/ml during late luteal phase (Day -4 to -2) in buffaloes that displayed obvious heat and silent estrus, respectively.

The present study examined the genetic polymorphism of this exon 10 in Egyptian buffalo FSHR gene. The primers used in this study flanked a 306-bp fragment from exon 10 of Egyptian buffalo FSHR gene. The PCR product of the primer specific for FSHR gene gave the specific band at size 306 bp (Fig. 1). These PCR amplified fragments of 306-bp were digested with *Alu*I endonuclease. Depending on the availability of the restriction site at position 243^244 (AG^CT), All buffaloes investigated in this study were genotyped as CC where DNA amplified fragments at 306-bp were digested with *AluI endonuclease* and gave two digested fragments at 243- and 63-bp (Fig. 2).

DNA sequence of 241 bp out of the amplified 306 bp was determined and also restriction site of the *endonuclease* enzyme pinpointed on the sequence chromatogram by the help of Finch TV DNA sequencing program (Fig. 3). The sequence alignment of 241 bp of Egyptian buffalo FSHR with published sequences (accession number: JX049145.1, Bubalus bubalis) was carried out using BLAST and showed that the 241 bp segment possess 100% identities (Fig. 4). The results also revealed that the sequence of the same DNA segment had 100% identities with the accession number: GenBank: EF650047.1, Bubalus bubalis (Fig. 5).

All buffaloes investigated in this study were genotyped as CC, where all experienced buffalo DNA amplified fragments at 306-bp were digested with

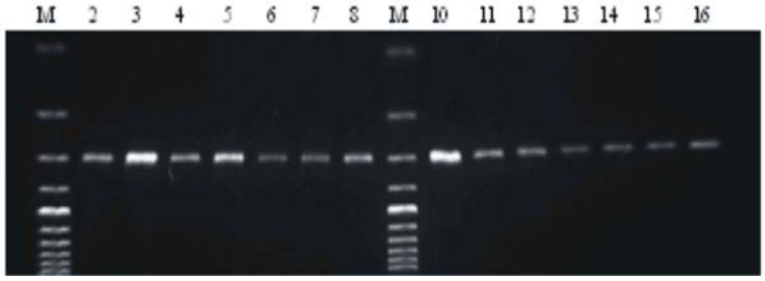

Fig. 1: DNA Ethidium bromide-stained gel of PCR products representing amplification of FSHR gene in Egyptian buffalo. **M**: 100-bp ladder marker. **Lanes** 2-8 and 10-16: 306-bp PCR products amplified from Egyptian buffalo DNA.

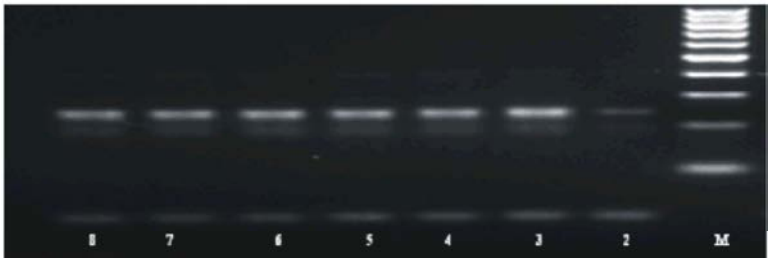

Fig. 2: The electrophoretic pattern obtained after digestion of PCR amplified buffalo *FSHR* with *AluI* restriction enzyme on electrophoretic gel. M: 100-bp ladder marker. Lanes 2-8: Homozygous CC genotypes showed two digested fragments at 243 and 63 bp.

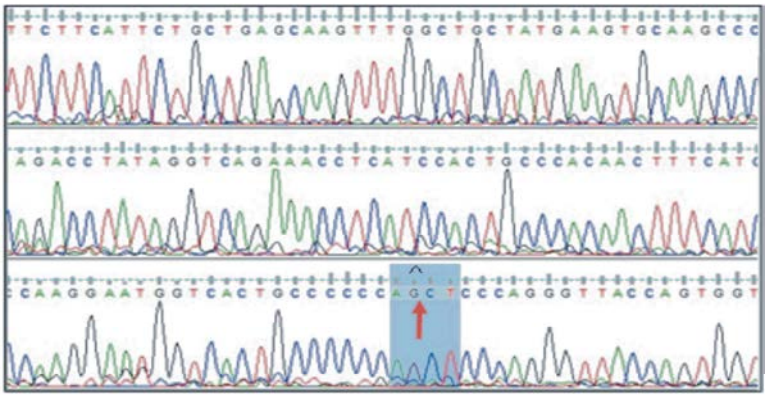

Fig. 3: DNA sequence chromatogram of DNA strand of FSHR gene of Egyptian buffalo. The arrow showed restriction site (**AG^CT**) of *AluI* on FSHR gene.

```
Query  3     GGGGGGCAGTGACCATTCCTTGGATGAAAGTTGTGGGCAGTGGATGAGGTTTCTGACCTA  62
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241   GGGGGGCAGTGACCATTCCTTGGATGAAAGTTGTGGGCAGTGGATGAGGTTTCTGACCTA  182

Query  63    TAGGTCTGGGCTTGCACTTCATAGCAGCCAAACTTGCTCAGCAGAATGAAGAAATCCCTG  122
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181   TAGGTCTGGGCTTGCACTTCATAGCAGCCAAACTTGCTCAGCAGAATGAAGAAATCCCTG  122

Query  123   CGGAAGTTCTTGGTGAAGATGGCATAGAGGAAGGGGTTGGCACAGGAGTTGATGGGGTAG  182
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   CGGAAGTTCTTGGTGAAGATGGCATAGAGGAAGGGGTTGGCACAGGAGTTGATGGGGTAG  62

Query  183   AACAGGACCAGGAGGATCTTTGACTTGGACACAGTGATGAGGGGCACCTTGAGGGAGGCA  242
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61    AACAGGACCAGGAGGATCTTTGACTTGGACACAGTGATGAGGGGCACCTTGAGGGAGGCA  2

Query  243   G  243
             |
Sbjct  1     G  1
```

Fig. 4: Sequence analysis of 241 segment of Egyptian buffalo FSHR amplified product compared to buffalo partial cds Sequence ID: **JX049145.1**

```
Query  3     GGGGGGCAGTGACCATTCCTTGGATGAAAGTTGTGGGCAGTGGATGAGGTTTCTGACCTA  62
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1166  GGGGGGCAGTGACCATTCCTTGGATGAAAGTTGTGGGCAGTGGATGAGGTTTCTGACCTA  1107

Query  63    TAGGTCTGGGCTTGCACTTCATAGCAGCCAAACTTGCTCAGCAGAATGAAGAAATCCCTG  122
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1106  TAGGTCTGGGCTTGCACTTCATAGCAGCCAAACTTGCTCAGCAGAATGAAGAAATCCCTG  1047

Query  123   CGGAAGTTCTTGGTGAAGATGGCATAGAGGAAGGGGTTGGCACAGGAGTTGATGGGGTAG  182
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1046  CGGAAGTTCTTGGTGAAGATGGCATAGAGGAAGGGGTTGGCACAGGAGTTGATGGGGTAG  987

Query  183   AACAGGACCAGGAGGATCTTTGACTTGGACACAGTGATGAGGGGCACCTTGAGGGAGGCA  242
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  986   AACAGGACCAGGAGGATCTTTGACTTGGACACAGTGATGAGGGGCACCTTGAGGGAGGCA  927

Query  243   G  243
             |
Sbjct  926   G  926
```

Fig. 5: Sequence analysis of 241 segment of Egyptian buffalo FSHR amplified product compared to buffalo partial cds. Sequence ID: **EF650047.1**

AluI endonuclease and gave two digested fragments at 243- and 63-bp.These results are similar to those found by Othman and Abdel-samad [16] in buffalo, whose primer pair yielded a 306-bp fragment for gene FSHR and use PCR-RFLP marker. On the other hand, Ahmed *et al.* [17] studied the genetic polymorphisms FSHR gene by using PCR-SSCP marker and establish their association with calving interval (CI) in buffalo. They found that the FSHR gene locus showed three polymorphic patterns with 61.4%, 21.4% and 17.2% for pattern 1, 2 and 3, respectively. Pattern 2 of FSHR gene locus recorded the lowest CI with expectation of 70% accurateness for the phenotype of high fertility.

The existence of allelic variants in FSHR gene reported in cattle [18-22] indicated that the FSHR gene is polymorphic. These deviations in the molecular structure of the FSHR gene results in desensitization of the FSHR receptors in the cell membrane which causes poor hormone signal transmission [23, 24]. By using *AluI endonuclease* for digestion of 306 bp product, it is easily differentiated between three different genotypes, depending on the presence or absence of the restriction site at position 243^244 (AG^CT), CC with two digested fragments at 243- an 63-bp, GG with three digested fragments at 193-, 63- and 50-bp and CG with four digested fragments at 243-, 193-, 63- and 50-bp.

Campagnari [25] investigated the polymorphisms of the FSHR gene by PCR-RFLP in various cattle breed composites. The polymorphism site analysis from digestion with *AluI* restriction endonucleases reported higher values for genotype GG (0.490). Likewise, Marson *et al.* [18] genetically characterized a population of European-Zebu composite beef heifers, using RFLP markers of FSHR gene. The verified genotypic frequencies varied from 0.075 to 0.347 (mean of 23%), 0.455 to 0.792 (mean of 58%) and 0.132 to 0.273 (mean of 19%) for genotypes GG, CG and CC, respectively, giving a greater incidence of heterozygosis for the greater part of the breed composition. Moreover, Marson *et al.* [19] studied the effects of *AluI* polymorphism of FSHR gene (exon 10) on sexual maturity in European-Zebu composite beef heifers from six diverse breeds. Three genotypes were identified (GG, CG and CC) with higher frequency of heterozygote in all examined breeds. The heterozygous heifers exhibited a greater pregnancy rate, but insignificant effects were noticed on the probability of pregnancy. Besides, variation in the bovine FSHR gene by using PCR-SSCP and DNA sequencing was investigated [26]. Yang *et al.* [26] verified one SNP of G278A located in the 5' upstream region of the bovine FSHR gene in Chinese Holstein cows. Cows with CC genotype had a substantial rise in the total number of ova ($P < 0.01$) and produced more transferable embryos ($P < 0.01$) than animals of the CD and DD genotypes.

CONCLUSION

The frequency of CC alleles in buffalo is very high. Monomorphic pattern of FSHR gene is considered a unique feature that may be added to the peculiarity of buffalo species. Accordingly, polymorphisms and its interaction with the fertility traits should be the subject of auxiliary exploration with enormous number of buffaloes.

ACKNOWLEDGMENT

This work was financially supported by a grant from Egyptian Academy of Science and Technology.

REFERENCES

1. Mishra, A.K., 1997. Application of biotechnologies to buffalo breeding in India. Bubalus bubalis, IV/97(Suppl.): 141-166.
2. Meyer, K., K. Hammond, P.F. Parnell, M.J. Mackinnon and S. Sivarajasingam, 1990. Estimates of heritability and repeatability for reproductive traits in Australian beef cattle. Livestock Production Science, 25(1–2): 15-30.
3. Themmen, A.P.N. and I.T. Hutaniemi, 2000. Mutations of gonadotropin and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary gonadal function. Endocrine Review, 2(5): 551-583.
4. Huhtaniemi, I. and J. Toppari, 2005. FSH Regulation at the molecular and cellular levels: Mechanisms of action and functional effects.In: Sertoli cell biology, Eds., Skinner, M.K. and M.D. Griswold. Elsevier Academic Press, pp: 155-165.
5. Houde, A., A. Lambert, J. Saumande, D.W. Silversides and L.G. Lussier, 1994. Structure of the bovine follicle-stimulating hormone receptor complementary DNA and expression in bovine tissues. Molecular Reproduction and Development, 39(2): 127-135.
6. Yarney, T.A., M.R. Sairam, H. Khan, N. Ravindranath, S. Payne and N.G. Seidah, 1993. Molecular cloning and expression of the ovine testicular follicle stimulating hormone receptor. Molecular and Cellular Endocrinology, 93(2): 219-226.

7. Robert, P., S. Amsellem, S. Christophe, J.L. Benifla, D. Bellet, A. Koman, A. and J.M. Bidart, 1994. Cloning and sequencing of the equine testicular follitropin receptor. Biochemical and Biophysical Research Communications, 201(1): 201-207.
8. Richard, F., N. Martinat, J.J. Remy, R. Salesse and Y. Combarnous, 1997. Cloning,sequencing and in vitro functional expression of recombinant donkey follicle stimulating hormone receptor: a new insight into the binding specificity of gonadotrophin receptorsJournal of Molecular Endocrinology, 18(3): 193-202.
9. Radwanska, E., J. Frankenberg and E. Allen, 1978. Plasma progesterone levels in normal and abnormal early human pregnancy. Fertility and Sterility, 30(4): 398-402.
10. Lussier, J.G., A. Houde, J. Ethier and D.W. Silversides, 1995. Bostaurus luteinizing hormone receptor mRNA. Genebank accession number:U20504.URL:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide.
11. Ahmed, A., S.P. Agarwal, V.K. Agarwal, S.A. Rehman and K.R. Laumas, 1977. Steroid hormones. Part II. Serum progesterone concentrations in buffaloes. Indian Journal of Experimental Biology, 15(8): 591-593.
12. Bachalaus, N.K., R.C. Arora, A. Prasad and R.S. Pandey, 1979. Plasma levels of gonadal hormones in cycling buffalo heifers. Indian Journal of Experimental Biology, 17(8): 823-825.
13. Pahwa, G.S. and R.S. Pandey, 1983. Gonadal steroid hormone concentrations in blood plasma and milk of primiparous and multiparous pregnant and non pregnant buffaloes. Theriogenology, 19(4): 491-505.
14. Batra, S.K., R.C. Arora, N.K. Bachalaus and R.S. Pandey, 1979. Blood and milk progesterone in pregnant and nonpregnant buffalo. Jounal Dairy Science, 62(9):1390-1393.
15. Mondal, S. and B.S. Prakash, 2002. Peripheral plasma progesterone concentrations in relation to oestrus expression in Murrah buffalo (Bubalus bubalis). Indian Journal of Animal Sciences, 73(3): 292-293.
16. Othman, E.O. and M.F. Abdel-samad, 2013. RFL polymorphism of three fertility genes in Egyptian buffalo. Journal of Applied Biological Sciences, 7(2): 94-101.
17. Ahmed, S.S., K.B. Abdel aziz, N.A. Hassan and D.M. Mabrouk, 2011. Genetic polymorphism of some genes related to reproductive traits and their association with calving interval in Egyptian buffalo. Genomics Quantitative Genetics, 3: 1-8.
18. Marson, E.P., J.B.S. Ferraz, F.V. Meirelles, J.C.C. Balieiro, J.P. Eler, L.G.G. Figueiredo and G.B. Mourao, 2005. Genetic characterization of European-Zebu composite bovine using RFLP markers. Genetics and Molecular Research, 4(3): 496-505.
19. Marson, E.P., J.B.S. Ferraz, F.V. Meirelles, J.C.C. Balieiroand and J.P. Eler, 2008. Effects of polymorphisms of LHR and FSHR genes on sexual precocity in a Bos taurus × Bos indicus beef composite population. Genetics and Molecular Research, 7(1): 243-251.
20. Almeida, S.E.M., M.S.N. Machado, S.N. Steigleder, C.L. Gama, M.H. Hutz, L.E. Henkes, J.C.F. Moraes and T.A. Weimer, 2000. Genetic diversity in Brazilian bovine herd based on four microsatellite loci. Genetics and Molecular Research, 23(2): 345-350.
21. Rahal, P., A.C. Latronico, M.B.F. Kohek, R.F.S. Lucia and M.P. Milazzotto, M.B. Wheeler, J.B.S. Ferraz, Eler J.P. and Garcia, J.F., 2000. Polymorphisms in the bovine follicle-stimulating hormone receptor gene. Animal Genetics, 31(4): 280-281.
22. Tambasco, D.D., M.M. Alencar, L. Coutinho, A.J. Tambasco, M.D. Tambasco and L.C.A. Regitano, 2000. Caracterizacao molecular de ani.J. mais da raca Nelore utilizando microssatelites e genes candidatos. Revista Brasileira de Zootecnia, 29(4): 1044-1049.
23. Gromoll, J., M. Simoni, V. Nordhoff, H. Behre, C. Geyter and E. Nieschlag, 1996. Functional and clinical consequences of mutations in the FSH receptor. Molecular and Cellular Endocrinology, 125(1-2): 177-182.
24. Huhtaniemi, I.T. and K. Aittomäki, 1998. Mutations of follicle-stimulating hormone and its receptor. Journal of Endocrinology, 138(5): 473-481.
25. Campagnari, F., 2002. Novas variantes moleculares dos genes dos receptores do hormonio liberador de gonadotrofinas (GnRHR) e do hormonio foliculo estimulante (FSHR) em femeas Bos primigenius indicus (Nelore). M.Sc. Thesis, Instituto de Biociencias, Universidade Estadual, Paulista, Campus de Botucatu, Botucatu, SP, Brazil.
26. Yang, W., S. Lib, S., K. Tanga, G. Huaa, C. Zhang, J. Yua, L. Hana and L. Yang, 2010. Polymorphisms in the 5' upstream region of the FSH receptor gene and their association with superovulation traits in Chinese Holstein cows. Animal Reproduction Science, 119(3-4): 172-177.