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Theriogenology

Genetic Monomorphism of Luteinizing Hormone Receptor (*lhr*) Gene by Restriction Fragment Length Polymorphism and Expression of LH in Egyptian Buffaloes

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ABSTRACT

The aim of this study was to identify luteinizing hormone receptor (*lhr*) genetic polymorphism by using PCR-RFLP and detection of LH level in serum, also to investigate their possible association with ovarian inactivity and silent heat in Egyptian buffaloes. Blood samples were collected with and without EDTA from female Egyptian buffaloes raised in small holder under the same managemental conditions. The present study was conductedon90 Egyptian river buffaloes from different locations in Menoufia governorate which were divided into three equal (n=30) groups according to their reproductive history and ultrasonographic examination (ovarian inactivity, silent heat and control). PCR amplification of DNA samples were revealed 303 bp product using specific primers and digested with HhaI restriction enzyme. The results were revealed that all animals were a monomorphic pattern at 303 bp and genotyped as *TT*, which indicates the fixation of *T* allele and absence of *C* allele, except one silent heat animal was showed heterozygous genotype *CT* 155, 148 bp (overlapped bands). While, the average of serum LH concentration was significantly higher (P<0.05) in silent heat (2.17 mIU/ml) and control groups (2.2mIU/ml) than ovarian inactivity group (1.99mIU/ml). In conclusion, no genetic polymorphism was detected for *lhr* gene of the three groups but, average LH concentration in serum was showed a significant difference within ovarian inactivity group.

Keywords: Buffalo, HhaI, LH, *lhr* and Serum.

INTRODUCTION

Numerous genetic, environmental, dietary, and management factors have an impact on the reproductive effectiveness of buffaloes (Kumar et al., 2014). Late maturation, inadequate estrous expression, anestrous, inactive ovaries, prolonged postpartum interval, seasonal cyclicity, and silent estrous all have an impact on buffaloes' reproductive performance (Sosa et al., 2016). The hypothalamus-pituitary-gonad axis and its relationships have a role in controlling reproductive function. Numerous genes affect physiological and endocrine processes that affect inherent fertility and overall production (Meyer et al., 1990). At the gonads, the follicular stimulating hormone (FSH) and luteinizing

hormone (LH) receptor (*fshr* and *lhr*) genes are expressed (Themmen and Huhtaniemi, 2000), being all of the members of the G protein coupled receptor family (Segaloff and Ascoli, 1993). The interstitial cells of the ovary and testis are stimulated by luteinizing hormone (LH), which is consists of an alpha and a beta subunit, LH is also important for the release of estrogen (Channing et al., 1980). The luteinizing hormone/choriogonadotropin receptor (LHCGR), which primarily mediates the cellular activities of LH, has features typical of receptors that work with G proteins, including a cellular domain, seven transmembrane domains, and an extracellular hormone-binding domain (Dufau et al., 1995). FSH, estrogen, and growth factors are responsible for triggering the expression of the *lhr* in the ovary (Mishra et al., 2003). LH triggers follicular wall rupture, ovulation, and the release of progesterone from the corpus luteum (CL) (Terzano et al., 2012). For example, LH interacts with *lhr*, affecting a number of processes that are essential for reproductive females to operate, including steroidogenesis, follicular development, oocyte maturation, ovulation, and corpus luteum production (Barros et al., 2010). Therefore, under physiological circumstances the presence of *lhr* on granulosa cells is essential for folliculogenesis from the acquisition of follicular dominance until ovulation (Ginther et al., 2001). The LH surge induces ovulation and promotes the fast and certain genes' transitory expression that have been demonstrated to be essential for ovulation in the granulosa cells of preovulatory follicles in a species-specific way (Richards, 1994). Any variation in the *lhr* gene's nucleotide sequences could affect the reproductive characteristics either directly or indirectly (Sosa et al., 2016). Furthermore, the *lhr*gene is suggested as a potential candidate gene for selecting buffaloes for breeding (Parmentier et al., 1999). LH secretion was lower during summer compared to winter season (Aboul-Ela and Barkawi, 1988). Additionally, it was noted that anestrus buffaloes in the summer did not show the ideal LH surge, despite the fact that most of the animals' LH levels rose from 3.06 to 8.67ng/ml (Razdan et al., 1981). Progesterone and PRL's inhibitory effects are thought to be the cause of the decrease in LH levels (Singh and Chaudhry, 1992). Furthermore, Low LH levels are thought to be induced by LH stimuli, such as a feedback mechanism, rather than the pituitary's inability to produce LH (Batra and Pandey, 1982). The purpose of this study was to use PCR-RFLP assay to examine the status of *lhr* gene polymorphism in Egyptian buffaloes, with estimation of LH concentration in relation with some fertility problems like smooth inactive ovaries and silent heat.

MATERIALS AND METHODS

This study was assessed and agreed by the Animal Care and Welfare Committee Ethics, University of Sadat City, Egypt (Ethical approval number: VUSC-023-1-21).

Animals

The present study was conducted on a total number of 90 Egyptian buffaloes (1st to 4th lactation season ~3-8 years old) at Menoufia governorate, Egypt. These animals were raised in small holder farms at approximately similar managemental conditions. All animal cases were recorded properly, along with owner complaints. Ultrasonographic examination of all buffaloes was conducted through a trans-rectal ultrasonography (E1V SonoScape, ®China) with an endorectal probe of 8.6 MHz for detecting thepresenceor absence of CL on the ovary surface and the follicular development stage (Kandiel et al., 2013 and Othman et al., 2014). According to ovarian status after gynecological examination, animals were divided into three groups (30 animals for each group), the 1st group was suffering from ovarian inactivity, the 2nd group was

suffering from silent heat and the 3rd group was the control animals without any gynecological problems. All animals were apparently normal, vaccinated, dewormed and regularly fed a balanced ration.

Blood Sampling

Blood samples were collected from the jugular vein of each animal and divided into two tubes. The 1st tube was EDTA anticoagulant vacutainer tubes for genomic DNA extraction through using the Genomic DNA Mini Kit (Blood/Cultured Cell, Geneaid Biotech Ltd., ® Taiwan) in accordance with the directions provided by the manufacturer. The 2nd tube was non-heparinized vacutainer tube for harvesting of serum through 20 minutes of centrifugation at 3000 rpm, followed by labelling and storage at -20 °C prior to getting tested (Othman et al., 2014).

PCR Reaction and DNA Amplification

A303 bp fragment of the *lhr*gene's exon 11 was amplified using certain forward and reverse primers5' CAA ACT GAC AGT CCC CCG CTT T 3'and 5'CCT CCG AGC ATG ACT GGA ATG GC 3', respectively (Othman and Abdel-Samad, 2013).

Amplification reactions were made with a final volume 12.5µl for each sample (6.25 µl PCR master mix (Thermo Fisher Scientific Inc. USA), 1 µl forward primer, 1 µl reverse primer, 2.25 µl distilled water (DW) and 2 µl DNA sample).A preliminary denaturation at 94°C for 5 minutes was followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The 100 bp DNA ladder was used to compare the size of the amplified product. The PCR product was checked on 3% agarose gel electrophoresis in 0.5X Tris Acetate EDTA Buffer(TAE) after staining with Ethidium Bromide (EtBr) and visualized using E-Gel Imager(SKU: 10157, Life Technologies, The Lab World Group, United States).

Restriction Fragment Length Polymorphism (RFLP) Technique

For genotyping, the restricted digestion occurred at 37 °C (Dry Bath Incubator-MK200-2, Hangzhou Allsheng Instruments Co., Ltd., Zhejiang) for 15 minutes in a total volume of 15µl containing 10 µl of PCR product, 1.5 µl of 10X RE (rCutSmart) buffer, 0.5 µl HhaI enzyme (10 Units) (New England Biolabs, Inc.) and 3 µl DW. For identifying the genotypes, digested samples (10 µl) were separated on a 3% agarose gel containing EtBr at 2 V/cm for 1 h.

Estimation of LH concentration in serum

Serum level of LH reproductive hormone was determined using highly specific ELISA Kits (Fine Test®, Fine Biotech Co., Ltd., Wuhan, China) according to Hameed and Alsalim et al. (2022).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) by SPSS program version 19for Windows (IBM, Armonk, NY, USA).

RESULTS

The *lhr* gene's amplified PCR products (exon 11) were run on a 3% agarose gel and observed using UV transilluminator. All the PCR products (303 bp) of *lhr*/ HhaI were undigested and found to be monomorphic in nature in all animals of the 1st and 3rdgroups (ovarian inactivity and control group) and revealed the existence of an uncut banding pattern (*TT* genotype) at position 303 bp (Fig.1, 2).

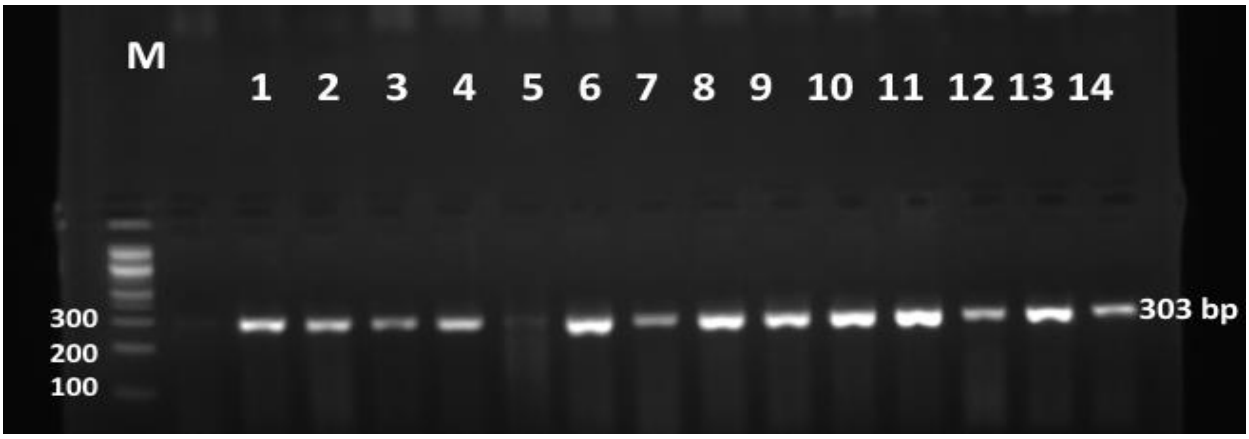

Fig (1): 3% agarose gel showed DNA electrophoresis pattern was obtained after digestion of PCR amplified *lhr* gene with HhaI restriction enzyme (lane 1-14), M 100 bp ladder marker of 1st group.

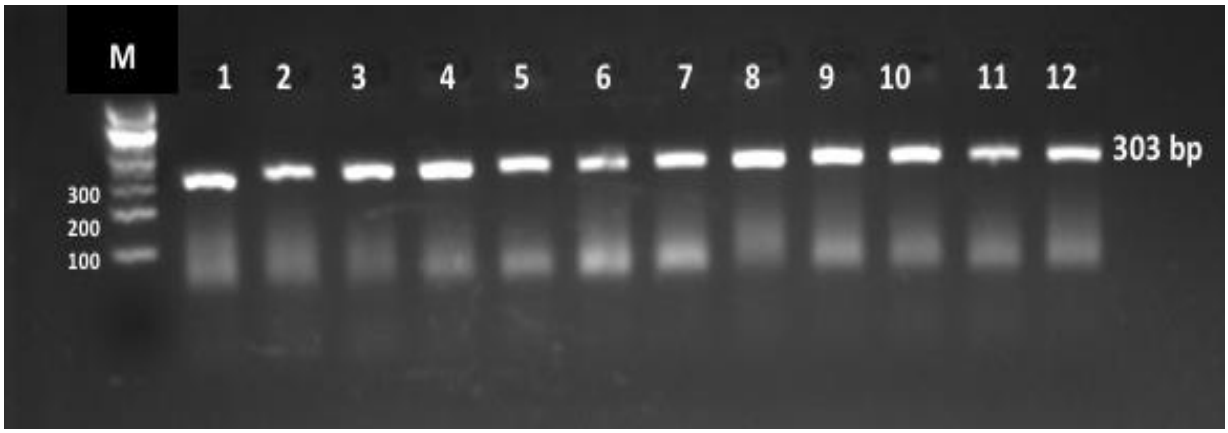

Fig (2): 3% agarose gel showed DNA electrophoresis pattern was obtained after digestion of PCR amplified *lhr* gene with HhaI restriction enzyme (lane 1-12), M 100 bp ladder marker of 3rd group.

The animals in the 2nd group (silent heat),the 303 bp PCR product remained undigested by HhaI restriction enzyme except, the heterozygous genotype *CT*155, 148 bp (overlapped bands) was detected only in one animal lane 2 (Fig. 3).

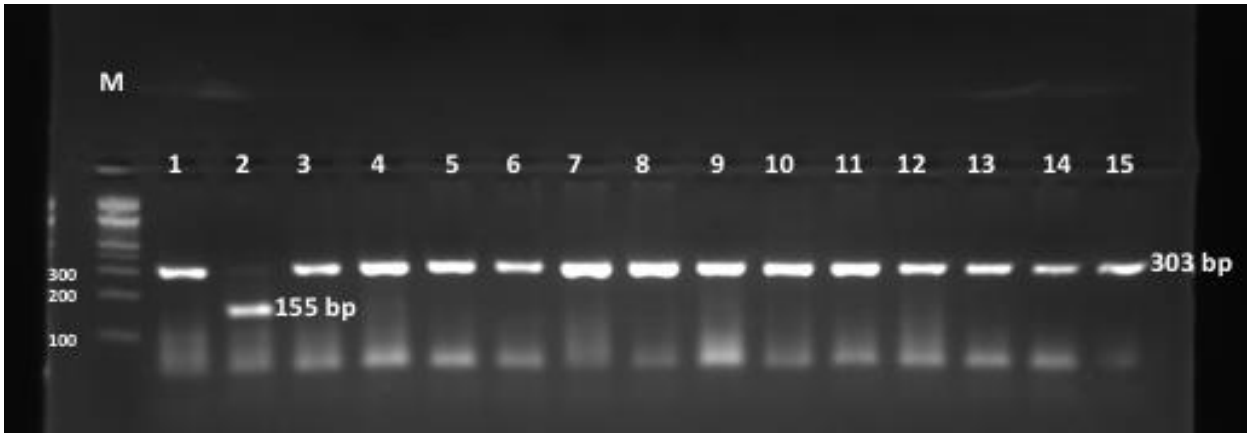

Fig (3): 3% agarose gel showed DNA electrophoresis pattern was obtained after digestion of PCR amplified *lhr* gene with HhaI restriction enzyme (lane 1-15), M 100 bp ladder marker of 2nd group.

The average concentration of LH in 1st group, 2nd group and 3rd group were recorded as (1.98 ± 0.088, 2.17 ± 0.057 and 2.2 ± 0.068) mIU/ml, respectively (Table1).The concentration of serum LH was showed a significant increase in the 2nd and 3rd group than the 1st group (Fig. 4).

Table (1): The concentration of LH in serum samples:

Animal's groups	**Concentration of LH in serum(mIU/ml)** **Mean ± SE**
1st group (Ovarian inactivity)	1.98 ± 0.088^{b}
2nd group (Silent heat)	2.17 ± 0.057^{a}
3rd group (Control)	2.2 ± 0.068^{a}

a and b: The concentration of LH in serum denoted with the same column with different superscripts are significantly different at ($P<0.05$) when $F=12.64$.

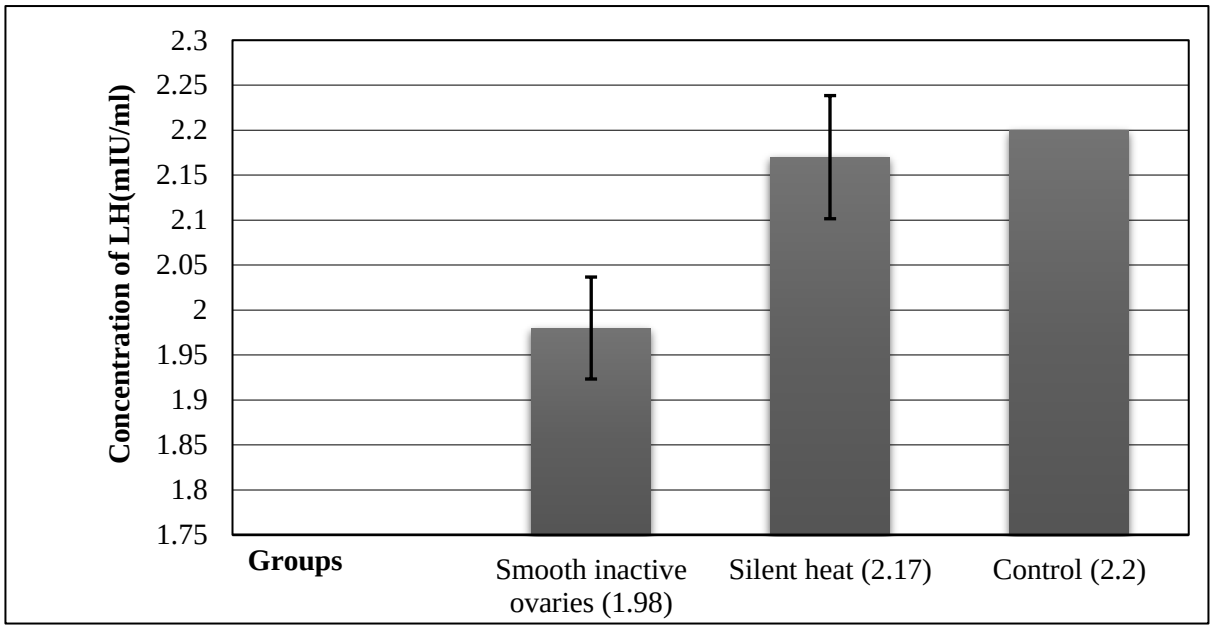

Fig (4): Concentration of LH (mIU/ml)in the different groups.

DISCUSSION

LH interacts with *lhr*, influencing various factors that are essential for female reproductive function, involves the development of the corpus luteum, ovulation, oocyte maturation, and steroidogenesis, as well as follicular development (Barros et al., 2010). In this study, the primers for *lhr* gene was amplified DNA fragments at303 bp. This result agrees with Othman and Abdel-Samad (2013) in buffaloes. DNA polymorphic markers can be used to determine an individual genotype at any locus, provide information about or support for allele frequencies, and help in marker-assisted selection.This study was found an uncut banding pattern (*TT* genotype) at position 303 bp. We could not identify any animal with homozygous *CC* 148 bp and heterozygous *CT* (155and 148 bp) genotypes while, we only identify one silent heat animal with heterozygous *CT*155, 148 bp. Similar findings were reported by Sosa et al. (2016) in Egyptian buffaloes and Kumar et al. (2014) in Murrah buffaloes. In European-Zebu composite cattle, *TT, CT* and *CC* genotypes were also found, with genotypic frequencies of 5, 54, and 41%, respectively (Marson et al., 2005). The Bos tarus x Bos indicus beef composite population also had the same three genotypes (*TT*, *CT*, and *CC*) (Marson et al., 2008).

In the present study, the *CC* genotypes were absent in all samples. These results agreed with Kumar et al. (2014) in Indian Murrah/Graded Murrah buffaloes and Sosa et al. (2016) in Egyptian buffaloes. This study revealed that the frequency of *T* allele was 179 allele, while the frequency of *C* allele was only one. Similar allelic frequencies were reported in buffaloes (Othman and Abdel-Samad 2013; Sosa et al., 2016).Contrarily, the allelic frequencies of the *C* and *T* alleles were 45.8 and 45.2%, 56.3 and 43.7%, and 41.70 and 58.30%, respectively, for Kenana cows, Butana cows, and Erashy cows(Omer et al., 2016). So, the study was concluded the fixation of *T* allele in the selected buffaloes except, one animal from silent heat group was showed a poly morphic pattern at 155, 148 bp and genotyped as *CT*. Additionally, the *lhr*/HhaI locus was shown to be monomorphic in all tested animals from all groups. Moreover, Marson et al. (2005) reported that the genotypic frequencies for the *lhr* gene for genotypes *TT*, *CT*, and *CC*, respectively, ranged from 0 to 0.091,

0.366 to 0.849, and 0.151 to 0.574. Higher *TT* (0.540) values and lower *CC* values (0.030) were observed in the study of Milazzotto (2001) for a Nellore population. Furthermore, Yu et al. (2012) found that heifers with the *CC* genotype had significantly more transferable embryos than those with the *CT* and *TT* genotypes, and that heifers with the *CC* genotype had more total ova than those with the *TT* genotype. Due to the genetic differences amongst the animals, there were no other alleles present in this study than the *TT* allele.

Luteinizing hormone plays a substantial role in contributing ovarian inactivity in buffaloes (Razdan et al. 1981).In this study, the average concentrations of serum LH hormone samples were recorded in the 1st group (ovarian inactivity) (1.98 ± 0.088), 2nd group (silent heat) (2.17 ± 0.057) and 3rd group (control) (2.2 ± 0.068). These results were in contact with Agustina (2019) and Taher et al. (2020) who reported the reduction in both FSH and LH in inactive ovaries. Other related studies Janakiraman et al. (1980); Aboul-Ela and Barkawi (1988); Singh and Chaudhry (1992) recorded that the secretion was lower during summer compared to winter. It has been shown that ovarian inactivity and anestrum disorder during the summer are associated to lower LH levels and a lack of a preovulatory surge (Razdanet al., 1980). In contrast, Kaker et al. (1980) found that during estrus, both hot and cool months, mean peak LH concentrations were identical. Meanwhile, Abd El-Razekand Allam (2019) also disagreed with study results and reported that the serum concentrations of LH did not show a significant difference between animals with ovarian inactivity and control.

CONCLUSION

There is no genetic polymorphism in *lhr* gene between ovarian inactivity, silent heat and control groups while, the concentration of LH was decreased in ovarian inactivity group.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abd El-Razek, E.M.M. and Allam, T.S.2019. Some Biochemical Parameters and Hematological Picture in Cases of Smooth Inactive Ovaries in Buffalo-Cows. AJVS., 61(1): 83-92.

Aboul-Ela, M.B. andBarkawi, A.H. 1988. Pulsatile secretion of LH in cycling buffalo heifers as affected by season and stage of estrous cycle. In: Proceedings of the 11th International Congress on Animal Reproduction and Artificial Insemination, 2, Dublin, Ireland, P. 3.

Agustina, W.K.K. 2019. Correlation between body condition score and hormone level of Bali cattle with postpartum anestrus. Indian J., of Anim., Res., 53(12), 1599-1603.

Barros, C.M., Ereno, R.L., Simões, R.A., Fernandes, P. andBuratini, J. 2010. Use of knowledge regarding LH receptors to improve super

stimulatory treatments in cattle. Reprod.,Fertil., Dev., 22: 132-137.

Batra, S.K.and Pandey, R.S. 1982. Luteinizing hormone and oestradiol-17B in blood plasma and milk during the estrous cycle and early pregnancy in Murrah buffaloes. Anim.,Reprod., Sci., 5, 247–257.

Channing, C.P., Schaerf, F.W., Anderson, L.D. andTsafriri, A. 1980. Ovarian follicular and luteal physiology. Int., Rev., Physiol., 22: 117-201.

Dufau, M.L., Tsai-Morris, C.H., Hu, Z.Z. andBuczko, E. 1995. Structure and regulation of the luteinizing hormone receptor gene. J., Steroid.,Biochem., Mol., Biol., 53: 283-291.

Ginther, O.J.,Bergfelt, D.R., Beg, M.A. andKot, K. 2001. Follicle selection in cattle: Role of luteinizing hormone. Biol.,Reprod., 64: 197-205.

Hameed, W.S. andAlsalim, H.A. 2022.Ultrasonographical and hormonal comparative between true and postpartum anestrus of cows in south of Iraq. Int., J., of Health Sci., 6(S6), 7909–7925.

IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

Janakiraman, K., Desai, M.C., Anim, D.R., Sheth, A.R., Moodbird,S.B. and Wadadekar, K.B. 1980. Serum gonadotropin levels in buffaloes in relation to phases of estrous cycle and breeding periods. Indian J., Anim. Sci., 50: 601–606.

Kaker, M. L., Razdan, M.N. and Galhotra. M.M. 1980. Serum LH concentrations in cyclic buffalo (Bubalusbubalis). J.,Reprod.,Fertil.,60:419-424.

Kandiel, M.M., Gad, B.A., Sosa, G.A. and El-Azab, A.I. 2013. Follicular dynamics and uterine status after synchronization of ovulation in early post-parturient Egyptian buffaloes. Buffalo Bulletin. 3: 165-181.

Kumar, R., Gupta, M., Shafiq, S., Balhara, A.K., Sadeesh, E.M. and Singh, I. 2014.Luteinizing hormone receptor gene polymorphism and its association with post-partum anestrus in Murrah buffaloes. J., of Cell and Tissue Res., 14(2):4237-4240.

Marson, E.P., Ferraz, J.B., Meirelles, F.V., Balieiro, J.C. andEler, J.P. 2005. Genetic characterization of European-Zebu composite bovine using RFLP markers. Genet., Mol., Res., 4: 496-505.

Marson, E.P., Ferraz, J.B.S., Meirelles, F.V., Balieiro, J.C.C. andEler, J.P. 2008. Effects of polymorphisms of *lhr*and *fshr*genes on sexual precocity in a *Bos taurus*× *Bos indicus* beef composite population. Genet., and Mol., Res., 7(1):243-251.

Marson, E.P., Ferraz, J.B.S., Meirelles, F.V., Balieiro, J.C.C., Eler, J.P. andFigueiredo, G.G. 2005. Genetic characterization of European-Zebu composite bovine using RFLP markers. Genet., and Mol., Res., 4(3):496-505.

Meyer, K., Hammond, K., Paenell, P.F., Mackinnon, M.J. andSivarajasingam, S. 1990.Estimation of heritability and repeatability for reproductive traits in Australian beef cattle. Livestock Prod., Sci., 25(1-2):15- 20.

Milazzotto, M.P. 2001.Mutacoes no gene do receptor do hormonluteinizante (*lhr*) bovinoeassociacao com precocidade sexual emfemeasBosprimigenius indicus (Nellore). M.Sc. Thesis, Instituto de Biociencias, UniversidadeEstadualPaulista, Botucatu.

Mishra, S., Lei, Z.M. and Rao, C.V. 2003. A novel role of luteinizing hormone in the embryo

development in co-cultures. Biol.,Reprod., 68: 1455-1462.

Omer, N.N., Gornas, N., Rahmatalla, S.A. and Ahmed, M.K.A.2016. Genetic characterization of indigenous Sudanese cattle using *fshr* and *lhr* genes. American Sci., Res., J., for Engineering, Tech., and Sci., 24(1):1-9.

Othman, E.O. and Abdel-Samad, M.F. 2013. RFLPolymorphism of three fertility genes in Egyptian buffalo. J., of Appli., Biosci., 7(2):94-101.

Othman, E.O., Wahid, M.A., Balabel, A.E.,Zaabal M.M., El Khadrawy, H.H., and Hanafy, E. 2014. Genetic Polymorphism of *Cyp19* Gene and its Association with Ovarian Activity in Egyptian Buffaloes. Glob., Vet., 12 (6): 768-773.

Parmentier, L., Portelle, D., Gengler, N., Prandi, A., Bertozzi, C. andVleurick, L. 1999. Candidate gene markers associated with somatotropic axis and milk selection. Domes., Anim., Endocrinol., 17:139-148.

Razdan, M.N.Kakar, M.L. andGalhotra, M.M. 1981. Serum luteinizing hormone levels of non-cycling buffaloes (*Bubalus bubalis*). Indian J., Anim., Sci., 51, 286–288.

Richards, J.S. 1994. Hormonal control of gene expression in the ovary. Endocrinol., Rev., 15: 725-751.

Segaloff, D.L. and Ascoli, M. 1993.The lutropin/choriogonadotropin receptor 4 years later. Endocrinol., Rev., 14: 324-347.

Singh, B. and Chaudhry, K.C. 1992. Plasma hormonal and electrolyte alterations in cycling buffaloes (Bubalusbubalis) during hot summer months. Int., J.,Biometereol., 36, 151– 154.

Sosa, A.S.A., Mahmoud,K.Gh.M., Kandiel, M.M.M., Eldebaky, H.A.A., Nawito, M.F. and El-Roos, M.E.A.A. 2016.Genetic polymorphism of luteinizing hormone receptor gene in relation to fertility of Egyptian buffalo. Biotech., Indian J., 12(5):1-11.

Taher,K.J., Khamees, H.A. and Abbas, H.R. (2020). Clinical and Therapeutic Study of Postparturient Anestrous of Cow. Plant Arch., 20(1):1782-1784.

Terzano, G.M., Barile, V.L. and Borghese, A. 2012. Overview on reproduction endocrine aspects in buffalo. J., of Buffalo Sci., 1:126-138.

Themmen, A.P.N. andHuhtaniemi, I.T. 2000. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocrinol., Rev., 21: 551–583.

Yu, Y., Pang, Y., Zhao, H., Xu, X. and Wu, Z. 2012. Association of a missense mutation in the luteinizing hormone/choriogonadotropin receptor gene (LHCGR) with superovulation traits in Chinese Holstein heifers. J., of Anim., Sci., and Biotech., 3: 35.