Middle-East Journal of Scientific Research 25 (7): 1615-1618, 2017
ISSN 1990-9233
© IDOSI Publications, 2017
DOI: 10.5829/idosi.mejsr.2017.1615.1618

Genetic Polymorphism of Albumin Locus in Relation to Ovarian Activity in Egyptian Buffalo-Cows

Wahid M. Ahmed and Magdy M. Zaabal

Department of Animal Reproduction & AI, Veterinary Research Division,
National Research Center, Giza, Egypt

Abstract: Ovarian inactivity is one of the most important causes of low fertility in farm animals in most of developing countries including Egypt. It causes high economic losses due to low calf production and milk yield. A total number of 375 buffalo-cows reared at Lower Egypt governorates was investigated to evaluate the possible correlations between albumin locus as a gene marker and ovarian activity. Animal case history was registered and gynecological examination was carried out. Blood samples were collected for assaying serum progesterone and cortisol levels using ELIZA micro wells technique. Electrophoretic pattern of serum proteins were done using polyacrylamide gel. Gene frequencies were determined using Hardi-vainberg formula. Results revealed that 46.13% of examined buffalo-cows (173 out of 375) suffered from ovarian inactivity since more than 6 months after calving with very low progesterone level and high cortisol level in the blood serum as compared with those having normal ovarian activity. Immunogenetic investigations of serum proteins indicated that normal cyclic buffalo are characterized by high frequencies of Pr^A (0.742) and Pal^A (0.685) gene markers, while buffaloes with inactive ovaries showed high frequencies of Pr^B (0.615) and Alb^S (0.673) gene markers. It could be concluded that the desirable improvement in reproductive traits of buffalo-cows can be achieved through the application of biochemical and molecular genetic markers.

Key words: Polymorphism · Albumin Locus · Ovarian Activity · Egyptian Buffalo

INTRODUCTION

Reproductive disorders generally and inactive ovaries, especially are the main cause of low reproductive efficiency in buffaloes in most of developing countries. In Egypt, ovarian inactivity is the major obstacle for efficient reproduction in buffaloes, it leads to great economic losses and represents 52 – 85% of the reproductive failure [1].

Under optimal conditions, different stages of reproductive cycle is found to be influenced by genotype, nutrition, management and climate [2]. Ovarian inactivity is mainly manifested as late maturity or long postpartum anestrum and is directly or indirectly attributed to managemental, pathological and other external influences with consequent high economic losses [3-5]. This condition induces high economic losses due to decreased milk production, cost of treatment and decreased number of calves output during the animal life span, besides it predispose for infection of the genital system [6].

To improve reproductive efficiency in buffaloes several protocols of oestrous and ovulation synchronization have been adopted from their use in commercial cattle production. These protocols yield encouraging pregnancy rate of 30 to 50% which are comparable to those achieved in buffaloes bred at natural oestrus [7].

The used of sexed semen in buffalo heifers also showed promising pregnancy rates (50%) [8]. In recent years genetic markers such as morphological, chromosomal, biochemical and molecular are used to increase productivity of animals [9].

Biochemical markers have been tried out and these markers are often sex-limited, age depended and is significantly influenced by the environment. Molecular markers can be capable of detecting the genetic variation at the DNA sequence level have not only removed these limitations but also possess unique genetic properties [10].

Corresponding Author: Wahid M. Ahmed, Department of Animal Reproduction & AI, Veterinary Research Division, National Research Center, Postal Code: 12622, Dokki, Giza, Egypt. E-mail: wahidmma@hotrmail.com.

The current study was carried out to clarify the possible relationships between albumin locus as a genetic marker and ovarian activity in Egyptian buffalo-cows.

MATERIALS AND METHODS

Animals: A total number of 375 non pregnant buffalo-cows was examined at veterinary clinics and small holder private farms at Lower Egypt governorates, Egypt. Animals that did not show oestrous cycle signs for a period of more than six months after calving were considered to be suffering from ovarian inactivity. Gynecological examinations aided by Ultrasonography (PiaMedical Flacse Saote, Netherland) with an endorectal array of 8.6 M Hertz were carried out twice for two successive weeks at least to register the reproductive status and/or disorder. Results were confirmed later on by progesterone levels.

Samples: Blood samples were collected and serum samples were separated by cool centrifugation (1300xg) then kept at -20°C until analysis of progesterone and cortisol levels.

Hormonal Assay: Plasma samples were examined for the concentration of serum progesterone [11] and cortisol [12] using ELIZA micro wells technique, using kits from Novotec, Germany and ELIZA reader (Anthos Zenyth 200rt). Sensitivity of assays were 2.0pg/ml and 0,025 µg/dl for progesterone and cortisol, respectively. Inter-run and intra-run precision coefficient of variations were 2.9 and 4.85 for progesterone and 5.17 and 4.70 for cortisol, respectively.

Electrophoresis and Estimation of Gene Frequency: Electrophoretic pattern of serum proteins were done using polyacrylamide gel [13]. Gene frequencies were determined using Hardi-vainberg formula ($P^2+2Pq+q^2=1$) after Mercoreva [14].

Statistical Analysis: Results were computed using SPSS program (ver.16.0). Data were statistically analyzed using student 't' test for values and Chi- Square for percentages according while, genetic equilibrium was determined by X^2 according to Snedecor and Cochran [15].

RESULTS

Results of the present study showed that ovarian inactivity still threats buffalo-cows reproduction in Egypt whereas 46.13% of investigated buffalo-cows was found to suffer from inactive ovaries (173 cases from total number 375) since more than 6 months after calving.

Table 1: Serum progesterone and cortisol levels in relation to ovarian activity in buffalo-cows. (Mean + SE)

	Normal Cyclic Buffalo-cows		
Hormone	Follicular	Luteal	Buffalo-cows with inactive ovaries
Progesterone (ng/ml)	0.58±0.09	4.21±0.52	0.10±0.6**
Cortisol(ug/dl)	1.31±0.23	1.40±0.35	2.68±0.26**

** P< 0.01

Table 2: Distribution of genotypes of Albumin locus (3 fractions) in relation to ovarian activity in Egyptian buffalo-cows

	Normal cyclic buffalo (N=202)			Buffalo with inactive ovaries N(173)		
Protein Loci	Genotypes	Gene frequencies	X^2	Genotypes	Gene frequencies	X^2
Prealbumin						
Pr	AA 120 (111.1)#	Pr^A (0.742)	10.04^{***}	AA 50 (25.5)	Pr^A (0.384)	61.7^{***}
	AB 60 (76.7)			AB 33 (81.7)		
	BB 22 (13.3)	Pr^B (0.257)		BB 90 (65.4)	Pr^B (0.615)	
Albumin						
Alb	FF 40 (57.2)	Alb^F (0.532)	23.7^{***}	FF 40 (18.4)	Alb^F (0.326)	55.6^{***}
	FS 135 (100.3)			FS 33 (75.9)		
	SS 27 (44)	Alb^S (0.467)		SS 100 (78.3)	Alb^S (0.673)	
Post albumin						
Pal	AA 108 (94.8)	Pal^A (0.685)	17.5^{***}	AA 77 (65)	Pal^A (0.613)	14.9^{***}
	AB 61 (86.9)			AB 58 (82.1)		
	BB 33 (19.9)	Pal^B (0.314)		BB 38 (25.9)	Pal^B (0.387)	

In brackets expected No of genotype *** P < 0.001

Table 1 reveals that progesterone level is mostly undetectable or low, while cortisol level was higher in buffalo-cows with inactive ovaries as compared to normal cyclic animals.

Table 2 shows that the most predominant gene markers of Albumin allele in normal cyclic buffalo are prealbumin (Pr^A 0.742) and postalbumin (Pal^A 0.685) , while buffaloes with inactive ovaries are distinguished by high frequency of gene markers (Pr^B 0.615) and (Alb^S 0.673).

DISCUSSION

In the present study, a total of 375 buffalo cows was investigated to detect the possible correlation between ovarian status and albumin locus as a genetic marker. It was found that 46.13% of investigated cows were suffered from inactive ovaries, especially during the dry season. These results were more or less the same as those previously reported by Hemieda [16] and Eltohamy *et al*. [17] among Egyptian buffalo. However, these results were higher than those reported by other authors [18, 19]. Differences in the reported incidence of ovarian inactivity may be related to managemental conditions [16, 18]. Genetic predisposition of the affection as indicated by appearance of unovular ovarian follicles in the ovary and chromosomal aberrations in the blood and splenic cells of affected animals can't be denied [20].

Low level of progesterone in present study was related to absence of functional luteal tissue [18]. On the other hand, the high cortisol level indicates the stressful conditions of the affected animal which affects the epizootic release of LH [16, 21].

In the present study fractionation of Albumin gave 3 fractions (Prealbumin (Pr), Albumin (A1) and post albumin (Pal). Using of blood protein loci as a genetic marker to evaluate reproductive efficiency were studied by many investigators [8, 10, 22, 23]. Albumin locus is controlled by an autosomal gene with two codominant alleles Alb^A and Alb^B since the six system studied are under mendelian genetic control, they can be used as genetic markers for water buffaloes (heterozygous genotype [22]. In buffalo blood protein polymorphism was used for breed identification and phylogenic studies. Giri and Pillai [24] were the first to report blood protein polymorphism in domestic water buffaloes.

In the present study, the most predominant alleles in normal cyclic buffaloes were prealbumin Pr^A 0.742 and Pal^A 0.685, while in buffaloes with inactive ovaries were showed high frequencies of Pr^B 0.615 and Albs 0.673 gene markers similar results were reported by Ahmed [25].

In recent years, the demonstration of genetic polymorphism at the DNA sequence level has provided a large number of markers techniques with variety of application [8, 23-30].

It was concluded that the desirable improvement in reproductive traits can be achieved through the application of biochemical and molecular genetic markers.

REERENCES

1. Salem, F.S., N.M. Mostafa, A.I. El Tawil, R.H. Youssef and M.Z. Abdel Aziz, 1974. Studies on some blood biochemical constituents in normal and abnormal cyclic buffaloes-Protein, lipid and transaminases. Proc 4th Wld Buffalo Congress, SaoPaulo, Brazil, pp: 200-201.
2. Jainudeen, M.R. and E.S.E. Hafez, 1993. Cattle and buffaloes in: E.S.E Hafez (Ed). Reproduction in Farm animals, Lea and Febiger, Philadelphia, 5th ed, pp: 315-329
3. Ahmed, W.M., 2013. Adverse conditions affecting ovarian activity in farm animals. Lambert Academic Publishing, Germany.
4. Perera, B.M.A.O., 2011. Reproductive cycles of buffalo. Animal Reproduction Science, 12: 194-199.
5. Akhtar, M.S., L.A. Lodhi, I. Ahmad, Z.I. Qureshi and G. Muhammad, 2012. Serum trace mineral variations in Nili-Ravi buffaloes suffering with prepartum vaginal prolapse in two different agro-ecological zones of Punjab, Pakistan. Theriogenology, 77: 1328-1333.
6. Ahmed, W.M., E.M. Hanafi and M.M. Zaabal, 2012. A trial to ameliorate the reproductive performance of native Egyptian cows suffering from reduced fertility. Global Veterinaria, 8(2): 174-178.
7. De Rensis, F. and F. Lopez, 2007. Protocols for synchronizing estrus and ovulation in buffalo (*Bublus bublis*): A Review. Theriogenology, 67(2): 209-216.
8. Warriah, H.M., D.M. McGill, R.D. Bush, P.C. Wynn and K.R. Chohan, 2015. A review of recent development in buffalo reproduction. Asian - Australian J. Anim. Sci., 28(3): 451-455.
9. Gaviraghi, A., R. Puglisi, D. Balduzzi, A. Severgnini, V. Bomaghi, G. Bongioni, A. Frana, L.M. Ganvini, A. Lukaj, C. Bonacina and A. Galli, 2013. Manimum number of spermatozoa per dose in Mediterranean Italian Buffaloes (*Bubalus bubalis*) using sexed frozen semen and conventional artificial insemination. Theriogenology, 79(8): 1171-1176.

10. Alim, M.A., D.X. Sun, Y.I. Zahng and M.O. Faruque, 2011. Genetic markers and their application in buffalo production. Journal of Animal and Veterinary Advances, 10(14): 1789-1800.
11. Wisdom, G.B., 1976. Competitive immunoenzymatic colorimetric method for quantitive determination of progesterone in serum or plasma. Clin. Chem, 22(8): 1243-1255.
12. Kowolaski, A. and W. Paul, 1976. Determination of cortisol by enzyme immunoassay. Clin.Chem., 25: 115.
13. Carlstorm, A. and B.G. Johnson, 1993. Electrophoresis immunofixation. Scand J. Immunology, 17: 23-30.
14. Mercoreva, E.K., 1977. Genetic Base of Selection in Farm Animals. Moscow, Coloc, pp: 121.
15. Snedecor, G.W. and W.C. Cochran, 1980. Statistical Methods. 7ED, Iowa State University Press, Iowa, U.S.A.
16. Hemieda, N.A., 1988. Reproductive problems in buffaloes in the world. Proc. 2nd Wld. Buff. Congr. India, 3: 197-205.
17. El Tohamy, M., W.M. Ahmed and A.S. Abdoon, 1994. Studies of reproduction in farm animals under the conditions of new reclaimed areas. Ovarian activity in relation to blood composition. Proc.4th Wld. Buff. Congr. Saopaulo-Brazil, 3: 612-614.
18. Dobson, H. and M. Kamonpatana, 1986. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and Zebu, J. Reprod. Fert., 77: 1-36.
19. Mansour, N.A., 1996. Histopathological studies on non-pregnant buffaloes and cows uterus and fallopian tube with special reference to ovarian finding. M.V.SC. Thesis (Theriogenology). Tanta University, Egypt.
20. Mahmoud, K.Gh, 1997. Some studies on fertility of buffaloes with reference to chromosomal aberration. M.Sc.. Vet, Theriogenology, Cairo University, Egypt
21. Ahmed, Y.F., 1987. Hormone profile in relation to some pathological reproductive changes in the buffalo cows PhD Vet. Thesis (Pathology), Cairo University, Egypt.
22. Tan, S.G., J.S.F. Barker, O.S. Selvaraj, T.K. Mukherjee and Y.F. Wong, 1993. Genetic studies of water buffalo blood markers. 1-red cell acid phosphatase, Albumin, catalase, Red cell Estrase-3, Group-specific component and protease inhibitor Biochemical Genetics, 31(5/6): 223-230.
23. Zaabal, M.M. and W.M. Ahmed, 2008. Monitoring of some reproductive parameters in local Egyptian Frisian cows with emphasis on the use of immunogenetic analysis for evaluation of fertility. Global J. Mol. Sci., 3: 1-6.
24. Giri, K.V. and N.C. Pilla, 1956. Multiple haemoglabins in the blood of animals. Nature, 178: 1057-1057.
25. Ahmed, W.M., 1997. Investigations on ovarian inactivity in Egyptian buffalo. Zag. Vet. J., 25(3): 94-102.
26. Michelizzi, V.N., M.V. Dodson, Z. Pan, M.E.J. Amaral, D.J. Michal, Mclean, J.E. Womack and Z. Jiang, 2010. Water Buffalo Genome science comes of Age. 1nt. J. Biol. Sci., 6(4): 333-349.
27. Gargani, M., L. Pariset, M.I. Soysal, E. Ozkan and A. Valentini, 2010. Genetic variation and relationships Among Turkish water buffalo populations. Animal Genetics, 41(1): 93-96.
28. Othman, E.O., W.M. Ahmed, E.A. Blabel, M.M. Zaabal, H.H. El-Khadrawy and E.M. Hanafy, 2014. Genetic polymorphism of cypl 9 gene and its Association with Ovarian Activity in Egyptian buffaloes. Global Veterinary, 12(6): 768-773.
29. Mancini, G., T. Biagini, G. Chillemi, F. strozzi, J. Williams, S. Schorder and A. Zimin, 2012. Status of the buffalo Genome. Plant and animal genome San Diego. US (http://pag.confex.com/pag/xx/ webprogram/paper 1541.html.
30. Elnahas, S.M., A.H. El-Kassas, A.A. Abu Mossallam and M. Warda, 2017. A study on IL8RB gene and polymorphism as a potential immuno-compromised adherent in exaggeration of partentral and mammocrine oxidative stress during mastitis in buffalo. J. Adv. Res., 8(6): 617625.