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Genetic Constitutions Affecting Ovarian Activity in Egyptian Cattle and Buffaloes

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Abstract: The present work was carried out to investigate genetic constituents affecting ovarian activity in Egyptian cattle and buffaloes. From animals coming to veterinary clinics at Al Sharkia governorate, Egypt, during the period from September 2008 to March 2009, 20 heifers and 20 polyparous cows were selected from cattle and buffaloes. All animals were clinically and gynecologically examined through rectal palpation of genital organs aided by ultrasnography. Animals showing no physiological structures on ovaries were considered as non cyclic. Also, the ovarian activity was confirmed later on by assaying of serum progesterone levels using ELISA microwell technique. Blood serum protein polymorphisms were analyzed using polyacrylamide gel electrophoresis. Six blood protein loci; pre-albumin, albumin, transferrin, post-transferrin, α-globulin and amylase were investigated. Results indicated that in cattle, high frequency of Al^{A}, Tf^{A}, Ptf^{B} and $F_2\alpha^{A}$ gene markers were detected in cyclic heifers, while high frequency of Al^{B} and Tf^{D} gene markers were detected in heifers suffering from delayed puberty. In buffaloes, Al^{F} and $F_2\alpha^{B}$ gene markers were predominant in normal cyclic heifers and Tf^{D} predominated in animals suffering from delayed puberty during the breeding season. It polyparous cows with normal functioning ovaries, high frequency of Al^{A}, Pal^{A}, Tf^{A}, Ptf^{A}, $F_2\alpha^{A}$ and $Am1^{B}$ gene markers were detected, while Pr^{B}, Al^{B}, Pal^{B} and $S_2\alpha^{B}$ gene markers predominated in cows having non functioning ovaries. In buffaloes, Pr^{A}, Al^{F}, Pal^{A}, Tf^{D}, Ptf^{A} and $F_2\alpha^{A}$ gene markers predominated in cyclic polyparous animals, while Al^{S} was more frequent in non-cyclic polyparous buffaloes. Serum progesterone level of heifers averaged 3.57 ± 0.93 and 2.71 ± 0.38 and of cows, 4.91 and 3.66 ng/ml in cattle and buffaloes during the mid luteal phase of the estrous cycle, respectively. The level was undetectable (<0.02 ng/ml) in all animals suffering from delayed puberty and ovarian inactivity. It was concluded that ovarian activity is a polygenic trait that is controlled by Al^{A}, Tf^{A}, Ptf^{A} and $F_2\alpha^{A}$ genes in cows and Pr^{A}, Al^{F}, Pal^{A}, Ptf^{A}, $F_2\alpha^{A}$ and $F_2\alpha^{B}$ genes in buffaloes.

Key words: Buffaloes · Cows · Genetic constitution · Ovarian activity- progesterone level

INTRODUCTION

Buffaloes and cattle are the main source for meat and milk in Egypt. Ovarian inactivity is the main obstacle facing efficient production of these animals in Egypt, especially in small holder farms whereas; adverse environmental conditions such as malnutrition, bad hygiene, pollution and infection are prevailing [1].

It was reported that ovarian activity [2], reproductive performance [3] and *In vitro* oocyte fertilization [4] are somewhat lower in buffaloes as compared to cattle. Moreover, buffaloes are reputed for high incidence of reproductive disorders such as delayed puberty, silent heat, seasonality of breeding and long calving interval [5, 6].

There are many established factors that affect the reproductive performance of animals in term of breed, age, season, health condition and genetic constitutions [7]. Silent heat, cystic ovaries, metritis and retained placenta are the most frequent fertility-related disorders associated with heritability and genetic correlations during first lactation in cows [8].

Recently, blood protein polymorphism is used as immunogenetic analysis to evaluate the reproductive traits and fertility in farm animals. Most reproductive traits including calving interval and conception as well as reproductive disorders are determined by polygenic loci in farm animals. The correlation between genetic polymorphism and reproduction in buffaloes has been reported by Barwe *et al.* [9] and Lama *et al.* [10].

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Many studies identified consistent links between specific loci and reproduction. The cystic follicles in the ovary of buffalo-cows were highly linked to albumin and α-globulin loci [11]. The non pregnant Barki and Rahmany breeds of sheep showed a high frequency of Al^D during breeding season [7]. The early pregnancy was associated with Pal (BB), transferrin (DE) and post transferrin (AA) in buffalo-cows [12]. Dystocia was reported to be highly correlated to homozygotic genotypes; Al^A Tf^A, Am^A and Hb^A in Egyptian Baladi goats [13]. Single births were proved to be linked to high frequencies of Alb^A, Am^A, Cp^A and Hb^A in does [14].

The present study was designed to investigate genetic constituents affecting ovarian activity in heifers and polyparous Egyptian cattle and buffaloes.

MATERIALS AND METHODS

Animals: The present study was carried out on a number of 20 mature heifers (24 - 30 months old) and 20 polyparous animals (2 -3 calvings) from each of local Egyptian cattle and buffaloes. These subjects were selected from animals came to veterinary clinics at Al Sherkia governorate, Egypt during the period from September 2008 to March 2009. These animals were reared in small holder farms, fed on Barseem (during December - May), small amount of concentrate, crops residues and rice straw. A full case history and owner complain of each animal was recorded. Animals were clinically and gynaecologically examined through rectal palpation aided by ultrasonography using an Ultra sound apparatus (PiaMedical Falcs e`Saote, the Netherlands) with an endorectal linear array 6-8 MHz transducer according to Terzano [15] to register the status of the reproductive organs.

Collection of Blood Samples: Blood samples were collected from jugular vein and serum was separated after coagulation by centrifugation (x 3000 g, 15 minutes at 4°C) and kept at -20°C for different analyses.

The Electrophoretic Analysis: The electrophoresis of blood serum proteins was done using polyacrylamide gel electrophoresis according to Laemmali [16]. Genotyping and estimation of gene frequencies were performed according to Hardy- Weinberg law as outlined by Mercoreva [17].

Progesterone Assay: Serum progesterone level was assayed by ELISA microwell technique using kits from DIMA (Germany). The kit had a sensitivity of 2.0 pg /ml with inter-and intra-run precision coefficient of variations [18].

Statistical Analysis: Data were computed and statistically analyzed according to Sendecore and Cochran [19].

RESULTS

Genotyping of blood protein loci in heifers of cows and buffaloes in relation to ovarian activity was recorded in Table 1.

In cow- heifers, high frequency of Al^A, Tf^A, Ptf^B and $F_2\alpha^A$ gene markers were detected in cyclic heifers, while, high frequency of Al^B and Tf^D genes were detected in animals suffering from delayed puberty. In buffaloes, Al^F and $F_2\alpha^B$ gene markers were predominant in normal cyclic heifers and Tf^D predominated in animals suffering from delayed puberty during the breeding season.

In polyparous cows and buffaloes, the genotyping of blood proteins in relation to ovarian activity was recorded in Table 2.

It was evident that cows showing normal functioning ovaries have high frequency of Al^A, Pal^A, Tf^A, Ptf^A, $F_2\alpha^A$ and $Am1^B$ gene markers, while Pr^B, Al^B, Pal^B and $S_2\alpha^B$ gene markers predominated in cows having non functioning ovaries. In buffaloes, the distribution of different genotypes indicated that Pr^A, Al^F, Pal^A, Ptf^A and $F_2\alpha^A$ gene markers predominated in cyclic polyparous animals, while Al^S was more frequent in polyparous buffaloes suffering from ovarian inactivity.

Progesterone levels in relation to ovarian activity in heifers and polyparous cows were shown in Tables 3 and 4, respectively.

In cattle, higher progesterone level was found in cyclic animals during the mid luteal phase of the estrous cycle in both heifers (3.57 ± 0.93ng/ml) and cows (4.91ng /ml). Meanwhile, the level was undetectable (< 0.02 ng /ml) in non heifers suffering from delayed puberty.

In buffaloes, progesterone levels averaged 2.71±0.38 and 3.66 ng/ml in cyclic heifers and cows during the mid luteal phase of the estrous cycle, respectively. The level was undetectable (<0.02 ng /ml) in non cyclic animals too.

Table 1: Blood protein genotypes in relation to ovarian activity in heifers of cows and buffaloes

	Cow-heifers (n=20)				Buffalo-heifers (n=20)			
	Normal Cyclic (n=10)		Delayed Puberty (n=10)		Normal Cyclic (n=10)		Delayed Puberty (n=10)	
Blood protein loci	GA	GF	GA	GF	GA	GF	GA	GF
Albumin	Al^A	0.821	Al^B	0.723	Al^F	0.739		
Transferrin	Tf^A	0.766	Tf^D	0.666			Tf^D	0.656
Post transferrin	Ptf^B	0.625						
α- globulin	$F_2\alpha^A$	0.713			$F_2\alpha^B$	0.716		

GA =GeneticAllele GF=Gene frequency

Table 2: Blood protein genotypes in relation to ovarian activity in polyparous cows and buffaloes

	Cow-heifers (n=20)				Buffalo-heifers (n=20)			
	Active ovaries (n=10)		Inactive ovaries (n=10)		Active ovaries (n=10)		Inactive ovaries (n=10)	
Blood protein loci	GA	GF	GA	GF	GA	GF	GA	GF
Prealbumin			Pr^B	0.737	Pr^A	0.838		
Albumin	Al^A	0.683	Al^B	0.681	Al^F	0.761	Al^S	0.654
Post-albumin	Pal^A	0.661	Pal^B	0.651	Pal^A	0.659		
Transferrin	Tf^A	0.580			Tf^D	0.722		
Post transferring	Ptf^A	0.701			Ptf^A	0.644		
α- globulin	$F_2\alpha^A$	0.666	$S_2\ \alpha^B$	0.618	$F_2\alpha^A$	0.864		
Amylase 1	Am^B	0.638						

Table 3: Serum progesterone level (ng/ml) in relation to ovarian activity in cow and buffalo heifers (Mean ±SEM)

Ovarian Activity	Cows	Buffaloes
Follicular phase	0.52 ± 0.10	0.46 ± 0.06
Mid luteal phase	3.57 ± 0.93	2.71 ± 0.38
Delayed puberty	<0.02	<0.02

Table 4: Serum progesterone level (ng/ml) in relation to ovarian activity in polyparous cows and buffaloes (Mean ±SEM)

Ovarian Activity	Cows	Buffaloes
Follicular phase	0.48 ± 0.06	0.49 ± 0.02
Mid luteal phase	4.91 ± 0.79	3.66 ± 0.84
Inactive ovaries	<0.02	<0.02

DISCUSSION

Poor reproductive efficiency is the most important obstacle for productivity in farm animals, especially buffaloes. Late sexual maturity, seasonal anoestrus and long periods of postpartum anestrum resulting in extended calving intervals cause great economic losses for livestock breeders [1, 20, 21].

In this study, six blood protein loci were analyzed and used as gene markers for predicting the possible correlation between genetic constitutions of cattle and buffaloes, either as heifers or as polyparous animals in one hand and their susceptibility to ovarian inactivity in the other hand. The results indicated that all these genetic loci are polymorphic in cows and buffaloes during different reproductive stages. This blood polymorphism was previously noticed in cattle, sheep and goats, indicating that many traits are controlled by multiple genes [8, 22, 23].

In cattle, the predominance of Al^A, Tf^A and Ptf^B in normal cyclic cows and $S_2\ \alpha^B$ in cows with inactive ovaries was in line with the result of Barakat *et al.* [24] in native Egyptian cows. Tf^D allele frequency in the present study was high in heifers suffering from delayed puberty. Few data are available regarding the interrelationship between genetic constituent and ovarian activity in heifers. However, This allele was known to be relevant to pathological disorders, whereas, Ahmed *et al.* [25] found high frequency of this allele in infertile Friesian cows following retention of fetal membranes. However, Hargrove *et al.* [26] reported high frequency of Tf^D associated with high conception rate in Holstein cattle. The predominance of Tf^A in cyclic heifers and polyparous cows agrees with the finding of Ahmed *at al.* [25] who indicated the same predominance of Tf^A in healthy cyclic Friesian cows. Serum transferrin locus had relationship to fertility, but results were often conflicting regarding identification of superior genotypes

or mating combination [27]. High frequency of Al^B was found in non cyclic heifers in the present study. In sheep, Zaabal *et al.* [28] reported that ewes suffering from ovarian inactivity showed high frequency of this allele. Variations in allele frequencies among breeds and different reproductive status could be attributed to the substantial genetic differences in ovulation rate, especially in multiple ovulating species [29, 30].

In buffaloes, the high frequency of Al^F and Tf^D gene markers in cyclic buffalo heifers was similar to those previously recorded by Lin [31], Osibova [32], Atula and Singh[33], Zheng *et al.*[34] and Shalaby *et al.* [35]. The predominance of Al^F, Pal^A, Ptf^A and Tf^D in normal cyclic buffalo-cows agrees with that of Zaabal [12], Shalaby *et al.* [35] and Osterhoff and Neethling [36]. The predominance of both Al^F and Tf^A in polyparous buffaloes is consistent with the findings of Zaabal [7] who reported high frequency of Al^F in Rahmany pregnant ewes and Tf^A in does giving multiple births for 3 successive breeding season.

In the present study, there was a relationship between Al^A and Tf^A in heifers and cows showing ovarian activity. In the same time, it was evident that Tf^D gene marker was associated with ovarian inactivity in cows and buffaloes. It was reported that cattle with high frequency of Tf^D homozygotes are unable to withstand nutritional and stress climatic conditions which cause greater changes in the blood proteins, hemoglobin and hematocrit in comparison with animals with Tf genotypes [36].

The high progesterone level recorded in buffalo-cows with high frequency of homozygotic genotypes; Pr^A, Al^F, Pal^A, Tf^D and $F_2\alpha^B$ was consistent with Osterhoff and Neethling [36] who indicated that Tf^D and $F_2\alpha^B$ were mostly predominant in buffalo-cows with BCS III and Zaabal [12] who indicated that the same previous genotypes were prevalent in normal cyclic buffalo-cows.

It could be concluded that active ovarian function is affected by Al^A Tf^A Ptf^B and $F_2\alpha^A$ genes markers in cow-heifers and Al^F $F_2\alpha^B$ gene markers in buffalo- heifers. In Polyparous animals, Al^A, Pal^A, Tf^A, Ptf^A, $F_2\alpha^A$ and $Am1^B$ gene markers affecting ovarian activity in cows and Pr^A, Al^F, Pal^A, Tf^D, Ptf^A, $F_2\alpha^A$ affecting the activity in buffaloes. The genetic analysis could be used for evaluation of ovarian activity in cattle and buffaloes. Attention should be paid for selecting animals for breeding purposes according to such genetic constitutions.

REFERENCES

1. Ahmed, W.M., G.M. Nabil, H.H. El-khadrawy, E.M. Hanafi and S.I. Abdel-Moez. 2006. Monitoring progesterone level and markers of oxidative stress in blood of buffalo-cows with impaired fertility. Egypt. J. Biophys. Biomed. Enging., 7: 71-83.
2. Daniel, B., 1987. Estrous behavior, ovarian morphology and cyclical variation on follicular system and endocrine pattern in water buffalo-heifers. DVM. thesis, Swedish University of Agri. Sci. Appsala, Sweden.
3. Hameida, N.A., 1988. Reproductive problems in buffaloes in the world. Proc. of 2 nd world buffalo Congress. EZ, Nagar, Indian, pp: 197-215.
4. Hegab, A.O., 1991. Studies on in vitro fertilization in cow and buffalo. M.V.Sc. (Theriogenology) Cairo Unviversity.
5. Bilal, M.Q. and A. Ahmed, 2004. Dairy hygiene and disease prevention. Usman and Bilal printing linkers, Faisalabad.
6. Ahmed, W.A. and K.S. Zaher, 2008. A field contribution on the relation between reproductive disorders and bovine diarrhea virus infection in buffalo-cows. Am-Euro. J. Agric. Environ. Sci., 3: 736-742.
7. Zaabal, M.M., 1997. Evaluation of some reproductive patterns of native breeds of sheep using blood protein genotypes and sex hormonal levels. Zag. Vet. J., 25(1): 60-66.
8. Heringstad, B., 2010. Genetic analysis of fertility-related diseases and disorders in Norwegian Red cows. J. Dairy Sci., 93(6): 2751-2756.
9. Barwe, V.K., M.M. Dhingra and S.S. Tomar, 1996. Factors affecting age at first calving and its genetic and phenotypic relationships with other reproductive traits in Gir cows. Indian J. Dairy Sci., 49(2): 100-104.
10. Lama, S.N., M.L. Del Lama, M.A. Mestrinen and N. Mortani, 1996. Estrase D polymorphism and partial characterization of red blood cell esterase in cattle and buffalo population. Brazilian J. Genetics, 19(2): 243-248.
11. Ahmed, W.M., S.I. Shalaby and M.M. Zaabal, 1998. Some biochemical constituents of preovulatory and cystic ovarian follicular fluids in buffalo-cows with emphasis on protein polymorphism. Inter. J. Anim. Sci., 13: 53-57.
12. Zaabal, M.M., 1998. Some biochemical and immunogenetical changes during early pregnancy in buffalo. 4Th Vet. Med. Zag. Congress. 26-28 August in Hurghada.

13. Zaabal, M.M., W.M. Ahmed and H.A. Sabra, 2001. Investigations on kidding in Egyptian Baladi goats with emphasis on immunogenetic structures and progesterone level. Egypt. J. Vet. Sci., 35: 71-87.
14. Zaabal, M.M., W.M. Ahmed and K.I. El-Ekhnawy, 2002. Relationship between twinning rate, some kidding parameters and immunogenetic markers in blood and colostrum proteins in Damascus does. Zag. Vet. J., 30(3): 74-83.
15. Terzano, G.M., 2005. Reproductive application of ultrasound in buffalo. In Buffalo Production and Research, Ed. Borghese, A. Monterotondo (Rome), Italy, pp: 137-144.
16. Laemmali, U.K., 1970. Structure of protein during the assembley of the head of bacteriophage T4. Nature, 227: 680.
17. Mercoreva, E.K., 1977. Genetic Base of Selection in Farm Animals. Moscow, Koloc, pp: 121.
18. Abraham, G.E., 1971. Radioimmunoassay of steroids in biological fluids. Acta Endocrinol., 75: 7.
19. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed., Iowa State Univ. Press, Ames, Iowa, USA.
20. Sharma, R.K., 2003. reproductive problems of buffaloes and their management. Compendium of lectures delivered to the Cent.Inst. Res. Buffalo, Hisar, India, pp: 119-126.
21. Ahmed, W.M., 2006. Adverse conditions affecting ovarian activity in large animals. Proc. the 3rd Intern. Conf. Vet. Res. Div. NRC, Cairo, Egypt, pp: 251-253.
22. Mwacharo, J.M., C.J. Oteino and A.M. Okeyo, 2002. Genetic variations between indigenous fat- tailed sheep populations in Kenya. S. Rumin. Res., 44: 173-178.
23. Nyamsamba, D., K. Nomura, M. Nozawa and M. Yokohama, 2003. Genetic relationship among native goat populations estimated by blood protein polymorphism. S. Rumin. Res., 47: 171-181.
24. Barakat, A.M., E.M. Hanafi, H.A. Sabra, M.M. Zaabal and W.M. Ahmed, 2001. Effect of parasitic infection on ovarian activity in native Egyptian cows and ewes with special reference to changes in some blood constituents and immunogenetic markers. Zag. Vet. J., 29(2): 121-136.
25. Ahmed, W.M., K.E. El-Ekhnawy, H.M. Desouky, M.M. Zaabal.and Y.F. Ahmed,1999. Investigation on retained fetal membranes in Friesian cows in Egypt. Egypt. J. Comp. Path. and Clinic. Path., 12(2): 160-177.
26. Hargrove, G.L., C. Kiddy, C.W.H. Young, A.G. Unter, G.W. Trimberger and R.E Mother, 1980. Genetic polymorphisms and milk and reproduction in Holstein cattle. J. Dairy Sci., 63(7): 2254-1166.
27. Kraay, G.J., 1970. Gene segregation at the FV and TF loci in cattle breeds. Anim blood group Biochem., Genet., 4: 119.
28. Zaabal, M.M., W.M. Ahmed and H.H. El-Khadrawy and K.A. El-Battawy, 2004. Studies on immunogenetic markers and amino acids concentrations in blood of Barki ewes in relation to ovarian activity. J. Egypt. Vet. Med. Assoc., 64(6): 65-77.
29. Fesus, L., R. Seibold, A. Al-Dabbag and E. Takacs, 1992. Estimation of genetic relationship among population of sheep. In: Genetic conversion of domestic livestock. Vol.2, Walingfird UK; CAB International, pp: 251-254.
30. Rendo, F., M. Iriondo, B.M. Jugo, L.I. Mazon, A. Aguirre, A. Vicario and A. Esttonb, 2004. Tracking diversity and differentiation in six sheep breeds from the north Iberian peninsula through DNA variation. S. Rumin. Res., 52: 195-202.
31. Lin, C.Y., 1987. Association of milk protein types with growth and reproductive performance of dairy heifers. J. Dairy Sci., 70: 29-39.
32. Osibova, G., 1988. Relationship between vital capacity and homozygosity of transferrin gene. In Selection of Farm animals Against Diseases. Vneblem, Moscow., pp: 19.
33. Atula, J. and A. Singh,1994. Association between serum protein polymorphic system and linear body measurements in Jersey cattle. Indian J. Dairy Sci., 47: 99-102.
34. Zheng, W.M., S.J. Lai and R.X. Shi, 1995. Serum albumin polymorphism of swamp buffalo in China. Chinese J. Anim. Sci., 31(1): 3-6.
35. Shalaby, S.I., W.M. Ahmed, M.M. Zaabal and H.A. Sabra, 1997. Studies on metabolic profile tests and plasma protein genotypes in normal cycling buffalo-cows as affected by body condition scores. J. Agri. Sci. Mansoura Univ., 22(9): 2821-2832.
36. Osterhoff, D.R. and L.P. Neethling, 1969. Recent studies on cattle transferrin. J. South African Med. Ass., 40: 75.