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Genetic Polymorphism of Immunoglobulin Loci in Relation to Ovarian Activity in Egyptian Buffalo-Cows

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Abstract: A total number of 375 buffalo-cows was studied to detect the relationship between ovarian activity and immunoglobulin loci as a gene markers. Polyacrylamid gel electrophoresis was used to investigate immunoglobulin fractions; α-globulin, transferrin and posttransferrin. Genotyping and estimation of gene frequency of each locus were estimated in relation to ovarian activity in buffalo-cows. Results showed that normal cyclic buffaloes are characterized by high frequency of $F\alpha_2^B$ (0.717) and Tf^D (0.792) alleles, while buffalo-cows with inactive ovaries are characterized by high frequency of Tf^E allele. Moreover, homozygotic genotypes were predominated in all studied animals, either with normal cyclic ovaries or with inactive ovaries. It could be concluded that immunoglobulin loci can be used as a gene marker in selection programs which are interested in the improvement of reproductive efficiency of buffalo especially during early ages for selection of superior genetic constitution animals.

Key words: Genetic polymorphism · Immunoglobulin · Ovarian activity · Egyptian buffaloes

INTRODUCTION

One of first goals in genetic selection programs, is to estimate the genetic potential during earliest ages. In this case, it is important to choose valuable economic characteristics such as growth performance, dairy yield and disease resistance. Among these early selection parameters are blood proteins. Serum transferrin and haemoglobin are two types among most important blood proteins [1].

Transferrin plays a very important role in physiological functions as well as in protection against microbial infection. Physiologically, iron is transported in blood by a plasma transport protein; transferrin in the form of a transferrin-iron complex and is transferred inside the cells via the transferrin receptor [2, 3].

Tsuji *et al.* [4] reported that transferrin stimulates the proliferation of fetal kidney rather than growth hormone or thyroxin, as other important roles of transferrin in addition to its well-known Fe- carrying function which is related to phylogenetical and ontogenetical characteristics and is restricted in cattle and water buffaloes.

The protective function of transferrin has been recorded in different animal species [5-8]. Martinez *et al.* [9] found association between genetic polymorphism in TLR4 gene and $NRAMP_1$ gene with susceptibility to brucellosis. Transferrin genotypes have been previously studied in Egyptian buffaloes in relation to ovarian activity [10-14].

Investigating proteins found in buffalo ovarian follicular fluid provide insight into follicular development processes and provide further understanding of intra-follicular maturation and oocyte quality. Some authors attributed the low quality buffalo oocyte due to the poor follicular microenvironment [15]. In this respect, Ahmed *et al.* [16] reported a correlation between some biochemical constituents of preovulatory and cystic ovarian follicles and transferrin genotypes in Egyptian buffalo-cows.

The main goal of the present study was to clarify the relationship between genotypes of immunoglobulin loci, especially transferrin and ovarian activity in Egyptian buffalo-cows where as majority of animal proteins are produced from these animals.

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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 . 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 at least twice for two successive weeks to register the reproductive status and /or disorder.

Samples: Blood samples were collected and serum samples were separated by cool centrifugation (Sigma, 2k15; 1300xg) then kept at -20°C until analysis .

Electrophoresis: Electrophoretic patterns of serum proteins were done using polyacrylamide gel electrophoresis (PAGE) according to Carlstrom and. Johnson [17] depending upon distribution of protein fractions according to their molecular weight following exposure to electric current .

Genotypin and Estimation of Gene Frequency: Genotyping was done according to distribution of autosomal alleles which controlling the appearance of the gene. Gene frequencies were determined according to Hardi-Vainberg formula (P2+2Pq+q2=1) cited after Mercoreva [18]. Where p2=frequency of homozygotic genotype AA and q2=frequency of homozygotic genotype BB.

Statistical Analysis: Results were computed using SPSS program (Ver.16.0). Data were statistically analyzed using Chi- Square [19].

RESULTS

Results of electrophoretic patterns of blood serum proteins including α- globulin ($F\alpha_2$), transferrin (Tf) and posttransferrin (Ptf) in buffalo-cow in relation to their ovarian activity are shown in Table 1.

Electrophoretic patterns showed that each of the studied loci are controlled by two autosomal alleles. Buffaloes with normal ovarian cyclic status were characterized by high frequency of $f\alpha_2^B$ (0.717) and (Tf^D) (0.792), while buffaloes with inactive ovaries were characterized by high frequency of transferrin Tf^E (0.601). Moreover, an interesting result in the present study was found and indicated that homozygotic genotypes are predominated in all studied animals, either with normal cyclic ovaries or with inactive ovaries.

DISCUSSION

A population is said to show polymorphism when two or more distinctly inherited varieties coexist in the some individuals [20]. This type of polymorphism is increasingly being used in the study of genetic variation within and between populations and to estimate the genetic divergence. This is because the biochemical elements (Blood proteins and enzymes) can be used as biomarkers of corresponding structural genes. These biomarkers are not affected and don't depend on environmental factors and this makes them suitable for genetic studies [21, 22].

Using of blood protein loci as a genetic markers to evaluate reproductive efficiency were previously studied [1, 6, 13, 15, 23, 24].

Ovarian inactivity is one of the most serious obstacle that causes great economic losses, in this respect many researchers studied the correlation between genetic constitution and Quantitative trait loci (QTL) especially reproductive parameters that has economic value [7, 9, 16, 25 - 27].

In the present study, distribution of genotypes of α-globulin, transferrin and posttransferrin were found to differ significantly from the expected proportions of homozygotes and heterozygotes. Moreover, results showed predominance of homozygotic genotypes in all studied loci in both cyclic animals and animals with inactive ovaries with high frequency of $F\alpha_2^B$ and Tf^D alleles in cyclic buffaloes and Tf^E in those of inactive ovaries. These finding agreed with those reported by Zayed and Zaabal [10], Shalaby *et al.* [11] and Ahmed *et al.* [16] especially for Tf^D allele, but did not agree with the finding of Ekblom *et al.* [2], Tsuji *et al.* [4], Majed *et al.* [28] and Amano *et al.* [29] who reported different gene frequency and the condition could be attributed to the breed differences. These results coincide with the finding of Galal *et al*. [30] for β-lacto globulin allele in Egyptian cattle and buffalo whereas, they reported that BB genotype = 0.917, but not agree with Amano *et al.* [29] who reported different figures. These differences have suggested that the polymorphism of blood proteins depends not only on the breed, but also on the breeding and mating methods [31, 32].

Table 1: Distribution of immunoglobulin genotypes and it's gene frequencies in relation to ovarian activity in Egyptian buffalo-cows

	Normal cyclic buffaloes (N=202)			Buffaloes with inactive ovaries N(173)		
Protein Loci	Genotyping	Gene frequencies	X^2	Genotypes	Gene frequencies	X^2
α -globulin (Fα2)	AA 37 (16.1)*	$F\alpha_2^A = 0.282$	52.7***	AA 80 (60.0)	$F\alpha_2^A = 0.589$	39.1***
	AB 40 (81.7)	$F\alpha_2^B = 0.717$		AB 44 (83.5)	$F\alpha_2^B = 0.410$	
	BB 125 (103.8)			BB 49 (29.0)		
Transferrin (TF)	DD 140 (126.7)	$Tf^D = 0.792$	32.6***	DD 55 (24.4)	Tf^D=0.398	80.7***
	DE 40 (66.2)	$Tf^E = 0.207$		DE 28 (82.7)	Tf^E=0.601	
	EE 22 (8.6)			EE 90 (62.5)		
Post transferrin Ptf	AA 71 (52.9)	Ptf^A=0.512	25.6***	AA 83 (56.6)	Ptf^A=0.572	67.3***
	AB 65 (100.7)	Ptf^B = 0.487		AB 32 (84.5)	Ptf^B=0.427	
	BB 66 (47.9)			BB 58 (31.5)		

*In brackets expected No of genotypes ***= P<0.001

From the finding of the current study, it could be concluded that immunoglobulin loci mainly transferrin locus can be used as a genetic marker in selection programs which are interested in the improvement of reproductive efficiency of buffalo especially during early ages for selection of superior genetic constitution animals.

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