Global Veterinaria 10 (4): 387-390, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.10.4.72154

Immunogenetic Investigations on Repeat Breeding Phenomenon in Purebred Arabian Mares

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Abstract: Investigations were carried out on 26 pure- bred Arabian mares to detect the genetic markers that may control the repeat breeding phenomenon in Arabian mares. Serum protein electrophoresis was carried out for genotyping blood protein loci and estimation of gene frequencies in both fertile and repeat breeder mares. Results indicated predominance of pr^N, $A1^F$, $F\alpha_2^A$ and E_S^H gene markers in repeat breeder mares and $F\alpha_2^B$, Es^G and Gc^F gene markers in regular breeder mares. It was concluded that there is a tight relation between genetic constitution and regularity of breeding process in purebred Arabian mares.

Key words: Arabian Mares · Gene Markers · Repeat Breeder

INTRODUCTION

The ability to apply the tools of horse genomics to problems in equine reproduction depends on two parameters. First, continuous development of mare dense linkage and comparative gene maps for the horse, with the establishment of a large bank of expression sequence tags (ESTs). Second, performing of sufficient trails of interest [1].

The relationships between genetic constitution and reproductive disorders in mares has been discussed in previous investigations [2-4]. Blood groups and blood protein loci have been used for evaluation of interbreed genetic diversity in horse breeds [5,6]. The protein variants occur by changes of amino acids or deletions that modify the molecular structure, being characters of simple Mendelian inheritance and co dominants. Such knowledge has special importance in studies of breed origin and formation, as well as complement to the parentage tests, increasing their reliability, and also as potential genetic markers for some characteristics of economic interest [7].

The current work investigated the immunogenetic constitution of Arabian mares through monitoring polymorphisms of some blood protein loci. Special interest was given to detect the association between the genetic constitution and the regularity of breeding in purebred Arabian mares.

MATERIALS AND METHODS

Experimental Mares: The present study was carried out on 26 purebred Arabian mares kept at Al-Zahara stud. Ain shams. Cairo. Egypt. Mares were divided into two equal groups. The 1st group included 13 Regular breeder, pregnant mares, while the 2nd group included 13 repeat breeder (Infertile) mares. Animal were housed in closed stables with open yard for exercises. Mares were fed on balanced ration; they have special care for diseases control, regular anti-parasitic drugs against external and internal health condition.

Evaluation of Fertility: Evaluation of fertility was depending upon:

- Case history.
- Detection of estrus using teaser stallion to monitor the reflex of the mare to the stallion [8].
- Rectal examination of the mares was done to display the physiological or pathological condition of the ovaries and tubular genitalia during the different stages of the reproductive cycle [9].

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Blood Sampling: Blood samples were collected from jagular vein into clean dry sterile and heparinized vacutainer tubes from both groups.

Analyses: Polyacrylamid gel electrophoresis (PAGE) was carried out [10] for genotyping of 5 blood protein loci:

- Albumin (Al)
- Pre-albumin (Pr)
- Transferrin (Tf)
- Alpha-globulin ($F\alpha_2$)
- Estrase (Es)
- Vitamin D binding –protein(Gc).

Estimation of gene frequency was carried out according to Hardi-Weinerg law [11].

- Statistical analysis of the collected data was done according to Spigal [12,13].

RESULTS

In the present study, regular breeder fertile mares revealing high frequency of the following alleles, $F\alpha_2^B$, Es^G and Gc^F, (Table 1) meanwhile, repeat breeder mares were distinguished by high frequency of Pr^N, Al^F, Tf^D, $F\alpha_2^A$ and Es^H alleles (Table 2).

Table 1: Genetic constitution of regular breeder purebred Arabian mares

Blood protein loci	---------- Genotyping ----------			Gene frequency	χ^2
Pre-albumin	NN	9	(8.3)*	Pr^N =0.498	0.9
Pr	NS	3	(4.2)		
	SS	1	(0.5)	Pr^S=0.501	
Albumin	FF	3	(3.2)	Al^F=0.500	
Al	FS	7	(6.5)		
	SS	3	(3.2)	Al^S =0.500	0.06
Transferrin	DD	5	(2.7)	Tf^D=0.460	
Tf	DO	2	(6.4)		
	OO	6	(3.8)	Tf^O =0.540	3.02
Alpha-globulin	AA	4	(1.9)	F α_2^A=0.380	
$F\alpha_2$	AB	2	(6.0)		
	BB	7	(4.8)	F α_2^B =0.610	5.9
Estrase	GG	8	(6.9)	Es^G =0.730	
Es	GH	3	(5.1)		
	HH	2	(1.0)	Es^H =0.270	2.03
Vit D	FF	11	(10.1)	Gc^F=0.880	
binding	FS	1	(0.2)		
Protein	SS	1	(2.5)	Gc^S=0.110	4.18
Gc					

N=13

*between brackets expected no of genotype

Table 2: Genetic constitution of repeat breeder purebred Arabian mares

Blood protein loci	---------- Genotyping ----------			Gene frequency	χ^2
Pre-albumin	NN	3	(1.5)	Pr^N =0.80	
pr	NS	3	(5.9)		
	SS	7	(5.5)	Pr^S=0.198	3.3
Albumin	FF	8	(6.2)	Al^F=0.692	
Al	FS	2	(5.5)		
	SS	3	(1.2)	Al^S =0.307	4.4
Transferrin	DD	6	(4.9)	Tf^D=0.615	
Tf	DO	4	(6.1)		
	OO	3	(1.9)	Tf^O =0.384	1.5
Alpha-globulin	AA	9	(6.9)	F α_2^A=0.730	
$F\alpha_2$	AB	1	(5.1)		
	BB	3	(0.9)	F α_2^B =0.269	9.7
Estrase	GG	2	(1.2)	Es^G =0.307	
Es	GH	4	(5.5)		
	HH	7	(6.2)	Es^H =0.692	1.00
Vit D	FF	5	(4.3)	Gc^F=0.576	
binding	FS	5	(6.3)		
Protein	SS	3	(2.3)	Gc^S =0.423	0.5
Gc					

N=13 *between brackets expected no of genotype

Moreover, in the regular breeder fertile mares, both Al and Tf loci have genetic equilibrium as indicated by the similar frequencies of both alleles in each loci.

DISCUSSION

Repeat breeding is an important reproductive disorder which causes great economic losses in farm animals. The repeat breeder animal is usually defined as sub-fertile animal which served three or more times and becomes not pregnant and continually returns to service in the absence of any obvious pathological disorder in the genital tract. The causes of this type of sub-fertility can be divided into two major categories; fertilization failure and early embryonic death [14].

The main goal of the present study was to characterize the immunogenetic constituents of purebred Arabian mare with emphasis on its relationships to regularity of breeding.

The genetic relationship between blood protein loci and reproductive performance is based on protein coding loci [15]. This fact get confirmed in the present investigation, whereas all studied loci were polymorphic., since the frequency of the most common allele was less than 0.95 [16].

Regular breeder fertile mares in the present study were characterized by high frequency of $F\alpha_2^B$, Es^G and Gc^F genetic alleles. The frequency of Es^G and Gc^F alleles in the present investigation is similar to the result obtained by Uzun *et al*. [17] and Sveistiene and Jatkauskiene [18].

Concerning Al locus, the frequency of its alleles was in line with the finding of Uzun *et al.* [17], Panepucci *et al*. [19] and Akyuz *et al*. [20]. However, this frequency was obviously different from the finding of, Diaz *et al*. [21] and Bowling. and Glark [22]. The phenotypes of Al loci in the present study (FF, FS and SS) are determined by two autosomal codominant alleles F and S. on the other hand, the I allele in Es locus was not found in the present study and this result is in contrast with the finding of Diaz *et al*. [21] who reported a high frequency of I allele in thoroughbred, Arabian and andalusian horses.

Some authors reported three alleles in the Al system; however only two alleles have been found in most studies [17, 22, 23].

Concerning Gc system, it is also controlled by two autosomal alleles F and S with high frequency of Gc^Fallele (0.88). This result is in line with those obtained by Sveistiene and Jatkauskiene [18] and Panepucci *et al.* [19] for Arabian horse and Weitkamp[24] for thoroughbreds.

Mares have repeat breeding status were characterized by high frequency of Pr^N, Al^F, $F\alpha_2^A$ and Es^H alleles. The high frequency of Pr^N in the present study is similar to the result detected by Abu-Atia *et al*. [25] and Pemberton *et al.* [26] and the condition may be attributed to the association between N haplotype of α-11 proteinase and endometritis [26]. On the other hand, Weitkamp *et al*. [27] confirmed the correlation between Tf and Es loci with endometritis in mares in the same time, it was reported that the Pr^N allele, is associated with prostaglandin receptor gene FPr [28].

Concerning α-globulin system, it was found that, $F\alpha_2^A$ allele is obviously frequent in repeat breeder mares (0.730). In the same time, it has been reported that mares with inactive ovaries are characterized by high frequency of Pr^N and $F\alpha_2^B$ (5) and the condition may be due to the polygenetic effect of these gene markers in ovulation in mares[29]. Moreover, this author reported the possible relationship between genetic constitution and physiological function whereas, the Pr system is responsible for thyroxin binding and transport in the body.

It was concluded that there is a tight relation between genetic constitution and regularity of breeding process in purebred Arabian mares. Special intrest must be paid for Gc^F and Pr^N alles, whereas high frequency of these allels are associated with regularity and repeat breeding phenomena in purebred Arabian mares,, repectively.This result must be take in consederation in the future breeding strategy planning.

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