

Monitoring of Gene Markers Associated with Fertility in Purebred Arabian Stallions

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Abstract: The present study was carried out on 28 purebred Arabian stallions, reared in a stud nearby, Cairo, Egypt. According to the breeding history, sexual behavior and clinical examination, stallions were divided into 2 groups : Fertile group (n=18) and infertile one (n=10). Blood samples were collected and serum samples were separated and electrophoretically analyzed for detection of gene markers of blood protein loci. Results showed that fertile stallions are characterized by high frequencies of Tf^D, Es^G and Gc^F gene markers with predominance of Es^H marker. Infertile stallions showed high frequencies of Al^F, Tf^O, Es^H, Ap^S and Gc^S gene markers with predominance of Gc^S marker. It could be concluded that fertility of purebred Arabian stallions is an important economic trait that could be used for selection in breeding programs.

Key words: Arabian Stallions • Fertility • Gene makers

INTRODUCTION

Fertility of stallions is influenced by significant number of genetic and environmental factors. Testicular biology including descending, size and consistency as well as sperm relevant characteristics including morphology, concentration and motility are detrimental factors for fertility in horses [1 - 4].

Worldwide, genetic factors associated with fertility of stallions had been established by many authors [4-9]. In the same time, infertility of stallions due to genetic causes has been reviewed by many investigators [6, 10-12].

Investigations on the relationship between genetic constitution and variation in quantitative traits are of perspective interest from both theoretical quantitative genetics and practical animal breeding points of views. This objective was studied through analysis of the reproductive performance of the stallion [5, 13, 14].

The use of recent molecular biology techniques for genome assembly can accurately indicate genetic factors controlling fertility in farm animals. Moreover, such technique can be of great help in the development of the novel tools for analyzing complex genetic traits [4,15].

The present study was carried out to detect some gene markers associated with fertility in purebred Arabian stallions in Egypt.

MATERIALS AND METHODS

Animals: The present study was carried out on 28 purebred Arabian stallions (aged 5-6 years) kept at Al-Zahraa stud, Ain Shams, Cairo, Egypt. Animal were housed in closed stables with open yard for exercise and they were fed on balanced ration consisted of barley and rice straw with green fodder (Barseem or Darraw). Special care for diseases control including regular application of antiparasitic drugs was routinely carried out.

Experimental Design: Animals were divided into 2 groups depending upon the breeding history, sexual behavior, clinical examination and farm pregnancy rate [16] :

- The first group included 18 normal fertile stallions, had no history of any breeding problems, with pregnancy rate up to 70% and healthy normal genital organs.
- The second group included 10 infertile stallions, having different testicular affections (unilateral cryptorchidism, testicular degeneration and orchitis) with pregnancy rate < 10%.

Blood Sampling: Blood samples were collected from jugular vein and serum was separated by centrifugation for 5 min at 3000 r.p.m.

Serum Electrophoresis: Electrophoretic patterns of serum protein were investigated as outlined by *Carlstrom and Johnson* [17]. Quantitation of different protein fractions was made using image densitometer (Bioral G 700).

Investigated Genetic Parameters

Gene Markers: Five serum protein loci were used as genetic markers including:

- Albumin (Al),
- Transferring (Tf)
- Estrase (Es),
- Alkaline phosphates (Ap)
- Vitamin D binding protein (Gc).

Gene Frequencies: Gene frequencies were counted as the expected Hardy- weiberg proportion of heterozygous genotypes in a particular phenotype class [18,19].

Heterozygosity Coefficient (SH): SH was estimated according to *Andersson and Davis*[19].

Statistical Analysis: The obtained data were statistically analyzed according to *Spiegel* [20].

RESULTS

Table 1 shows genotyping and gene frequency of the 5 studied blood serum protein loci in relation to fertility of purebred Arabian stallions.

Fertile stallions were characterized by high frequency of Al^F , Tf^D , Es^G , Ap^S and Gc^F gene markers with predominance of Es^G (Frequency 0.805). In infertile stallions, high frequency of Al^F , Tf^D , Es^H and Gc^S gene markers with predominance of Gc^S (Frequency 0.900) were recorded.

The coefficient of heterozygosity (SH) was higher in fertile (0.083) as compared to infertile (0.061) stallions.

DISCUSSION

The horse genome sequence was completed in 2006. This wealth of new data has already been exploited to provide some very powerful tools that can be used to define more simply inherited traits, especially those of economic importance in horse at the molecular level [21]. Moreover, The recent sequencing of the equine genome offers the possibility of both marker - assisted selection for fertility traits and more specific information about genetic mutation that may be associated with different levels of fertility in the stallion [11].

In the present study, the blood serum protein loci were used as gene markers to evaluate fertility status of purebred Arabian stallions in Egypt. It was found that all studied loci were polymorphic, this result was in line with the finding of *Jiskrova et al* [22] and *Vale et al.* [8].

Fertile stallions in the present study were characterized by high frequency of Al^F , Tf^D , Es^G , Ap^S and Gc^F gene markers with predominance of Es^G gene marker (0.805), while infertile stallions were characterized by high frequency of Al^F , Tf^D , Es^H , Ap^S and Gc^S gene markers with predominance of Gc^S gene marker (0.900).

Table 1: Genotyping and gene frequencies of serum protein loci of purebred Arabian stallion in relation to fertility

Genetic loci	Fertile stallions N= 18			Infertile stallions N=10		
	Genotypes	Gene frequency	χ^2	Genotypes	Gene frequency	χ^2
Albumin Al	FF 8 (6.7)*	$Al^F = 0.611$	1.60	FF 6 (4.9)	$Al^F = 0.7$	2.6
	FJ 6 (8.5)			FJ 2 (4.2)		
	JJ 4 (2.7)	$Al^I = 0.388$		JJ 2 (0.9)	$Al^I = 0.3$	
Transferrin Tf	DD 12(10.5)	$Tf^D = 0.777$	2.25	DD 2 (0.6)	$Tf^D = 0.25$	5.4
	DO 4 (6.2)			DO 2 (0.6)		
	OO 2 (0.9)	$Tf^O = 0.222$		OO 7 (5.6)	$Tf^O = 0.75$	
Estrase Es	GG 13(11.6)	$Es^G = 0.805$	4.40	GG 1 (0.2)	$Es^G = 0.15$	4.1
	GH 3 (6.6)			GH 1 (2.5)		
	HH 2 (2.7)	$Es^H = 0.194$		HH 8 (7.2)	$Es^H = 0.85$	
Alkaline phosphates Ap	FF 4 (2)	$Ap^F = 0.333$	4.50	FF 2 (1.2)	$Ap^F = 0.35$	1.1
	FS 4 (8)			FS 3 (4.5)		
	SS 10 (8)	$Ap^S = 0.667$		SS 5 (4.2)	$Ap^S = 0.65$	
Vitamine D binding protein Gc	FF 11 (7.9)	$Gc^F = 0.666$	10	FF 1 (0.1)	$Gc^F = 0.10$	10
	FS 2 (7.9)			FS 0.0 (1.8)		
	SS 5 (2)	$Gc^S = 0.333$		SS 9 (8.1)	$Gc^S = 0.90$	

* theoretical expected number of genotypes

The albumin system is well known polymorphic locus used in parentage control and pedigree analysis in equine [23]. However, the frequency of albumin in the present study revealed no difference between fertile and infertile stallions. In the same time, gene frequency of albumin in this study was similar to that recorded by Vale *et al.* [8] for Zemaitukai horse. However, transferrin locus, revealed 13 genotypes with high frequency of Tf^D in Zemaitukai horse, despite it revealed 6 genotype in investigated purebred Arabian stallion herein. On the other hand, Jiskrova [22] reported dissimilar results for albumin and transferrin in infertile stallions. Diaz *et al.* [24] in thoroughbred and Argentine Creal horses reported that these breed have 9 transferrin genotypes with frequencies of Tf^D and Tf^F similar to the present finding in fertile purebred Arabian stallions. In the same time, Al and Es gene markers of both thoroughbred and Argentine Creal horses were similar to those of the present investigated Arabian horses, for both gene frequencies and heterozygosity coefficient. The proportion of genetic variation could be attributed to breed differences.

In the present study, it was found that Esterase locus is controlled by two autosomal alleles G and H and this locus seems to have very important relationship with fertility in horses, whereas the allele G was predominant in case of fertile stallions while, the allele H was predominated in infertile ones. However, data reported by other authors for Estrase revealed the presence of 6 genotypes controlled by 3 autosomal alleles [8, 24].

Vitamin D binding protein Gc is considered as a fraction of post albumin protein and this fact explains the close relationship linkage between Al and Gc in the present study, whereas a close association was found between both loci herein. In the same time, the present frequency of Gc for fertile stallions was similar to the finding of Jiskrova *et al.* [22] with high predominance of Gc^F in the current study.

Data of the present study confirmed the autosomal, codominant inheritance at the five investigated serum protein loci for purebred Arabian stallions and this finding coincide with those of Fikretcelebi *et al.* [23], Ann and Oliver [25] andieria and Norma [26] and Anglika *et al.* [27].

In conclusion, fertility of purebred Arabian stallion is an important trait which is controlled by some genes, especially Es^G and Tf^D. During selection of breeding horses, such genetic constitution must be considered due to its high economic merits.

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