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Ovarian Activity in Purebred Arabian Mares in Relation to Some Blood Protein Gene Markers

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Abstract: The present study aimed to investigate the possible correlations between some genetic markers and ovarian activity in purebred Arabian mares. The study was carried out on 20 purebred Arabian mares kept at Al-Zahraa Stud, Ain Shams, Cairo, Egypt. Mares were divided into 2 groups according to ovarian status and the condition was confirmed by plasma progesterone level. Analysis of the blood protein system was used to study the genetic constitution of Arabian mares. Biochemical markers of four polymorphic loci (Al,Pal,Es and Ap) were electrophoretically identified. The Es^G and Ap^S loci appeared with high frequency in mares having active ovaries, while, the Al^J and Es^H loci showed high frequent in mares with inactive ovaries. In conclusion, ovarian activity of purebred Arabian mares is controlled by some gene markers Ap^S (0.77) ,and Es^G (0.64) and this fact must be taken into consideration in horses used for breeding purposes.

Key words: Gene Markers · Polymorphisms · Ovarian Activity · Purebred Arabian Mares

INTRODUCTION

Genetics is the main pillar for the selection of genetically superior animals that can pass their desired traits to their offspring. Molecular biology has led to the generation of techniques and knowledge that assist and complement the traditional system of genetic improvement [1]. Recently, there is a progress in the elucidation of factors regulating oocyte and follicle growth and development, as well as oocyte maturation, through the study of the crucial roles of a large number of proteins expressed throughout oogenesis, in particular during the early stages of folliculogenesis [2]. Some studies recorded the occurrence of significant relationship between the origin of mares from definite breeding lines and their reproductive results [3]. Over one thousand genes control the development of mammalian testes and ovaries, mutation in these genes can result in abnormal sexual development causing a range of reproductive abnormalities [4]. The relationship between genetic constitution and reproductive disorders in mares was mentioned in previous investigations [5, 6]. Blood groups and protein loci were used as markers for interbreed genetic evaluation of horses [7, 8] whereas14 variants of transferrin, 7 variants for estrarase, 3 variants for albumin and 18 variants for phospho-gluconate dehydrogenase loci were traced [9,10]. Also, elevation of alkaline phosphatase activity in uterine secretion was previously recorded as indicator in sub-fertile mares [11]. The main objective of the current investigation was to trace the possible variation in some blood protein genetic loci and its association to ovarian activity in purebred Arabian mares.

MATERIAL AND METHODS

Animals: The present study was carried out on 20 pure Arabian mares kept at AI-Zahra Stud, Ain Shams, Cairo-Egypt. Animals ranged in age from 3 to 21 years. They were housed in closed stables with open yard for exercise. Mares were fed on balanced ration consisted of barley and tibn with green fodder (Barseem or Darawa). Regular antiparasitic drugs against external and internal parasites and a daily training to improve general health condition were practiced.

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Based on the Reproductive Status, Mares Were Classified into 2 Groups as Follows:

Group1: Fertile mares (with active ovaries).

Group2: Infertile mares (with inactive ovaries).

Reproductive Aspects: Breeding record of mare was analyzed. External examination and rectal palpation for of the mares were done to display the physiological functions and pathological conditions of the ovaries and genitalia during the different stages of the reproductive cycle [12]. Estrus was detected using proven teaser stallion.

Blood Sampling: Blood samples were collected from the jugular vein into clean dry sterile and heparinized vacutainer tubes during estrus and diestrus from fertile and infertile mares. All collected blood samples were centrifuged and plasma samples were separated (X1500g at 4C° for 15 minutes) from heparinized tubes and kept at -20°C in sterile labeled stoppered Eppendorff vials till been used for the hormonal assay and the immunogenetic analysis.

Hormonal Assay: Plasma progesterone level was determined by ELISA kits (DIMA, Germany) using the micro- well method. The kit had a sensitivity of 2.0 pg/ml with the inter- and intra-run precision coefficient of variations of 2.9 and 4.85, respectively [13]

Immunogenetic Analyses: Electrophretic pattern of plasma protein was performed by polyacrylamid gel electrophoresis (PAGE) [14].

Genetic Markers: Genetic variants of some blood protein loci included, Albumin (A1), Post albumin (Pal), serum carboxyl esterase (Es), alkaline phosphatase (Ap) were investigated as genetic markers in the present study. Distribution of genotypes was done [15,16]. Determination of gene frequencies was estimated [15,17].

$P^2+2pq+q^2=1$

whereas:
P^2= homozygotic genotype AA
q^2= homozygotic genotype BB

RESULTS AND DISCUSSION

In the present investigation, ovarian activity of purebred Arabian mares was confirmed by plasma progesterone level, which was not detected (0.02 ng/ml) in mares with inactive ovaries, while it ranged between < 1 ng/ml (during the follicular phase) and > 3 ng/ml (during the luteal phase) in mares with active ovaries. Corpora lutea are the major source of progesterone in mares[12].

The analysis of four studied loci are present in Table 1. Al locus revealed three phenotypes (FF,FJ, jj) and they were determined by two autosomal codominant alleles F and J with frequencies 0.43 for Al^F and 0.57 for Al^J in mares with active ovaries, while their frequency were 0.33 for Al^F and 0.67 for Al^J in mares having inactive ovaries. Similar results were previously recorded by Ahmed *et al*. [5]. However, Pal locus revealed no significant difference in allele frequency. The Es locus revealed three phenotypes (GG, GH and HH) controlled by two co dominant alleles G and H with higher frequency (0.64) for Es^G allele in mare having active ovaries compared to Es^H which revealed high frequency in mares with inactive ovaries (0.67). In this respect it was reported that repeat breeder Mares were characterized by high frequency of Al^F and Es^H alleles [6]. Several studies were previously done on albumin and carboxyl esterase

Table 1: The distribution of genetic loci and gene frequencies of Arabian mares in relation to ovarian activity

		Active ovary		In active ovary	
Protein loci	Genetic alleles	Gene frequency	X^2	Gene frequency	X^2
Al	AlF	0.43	0.21	0.33	1.34
	AlJ	0.57		0.67	
Pal	PalD	0.57	0.21	0.50	0.37
	PalS	0.42		0.50	
Es	EsG	0.64	0. 32	0.33	1.34
	EsH	0.36		0.67	
Ap	ApF	0.23	1.45	0.50	0.37
	ApS	0.77		0.50	

genotyping in different lines of horse species and it was found that AB genotype is more frequent in albumin locus, while II genotype was dominant in Es locus. These results were not similar with the results of the present study and the condition may be attributed to the breed difference [8] whereas Arabian horse showed great difference than Lavaraderio species in the frequency of Al locus genotying whereas in Lavaradeiro horses the genetic allele Al^S was dominant (0.727) but in Arabian horse was (0.49) and this result is in line with the finding of present study for mares with active ovaries ($Al^J = 0.57$) [17].

Concerning Ap locus the present results revealed two autosomal codominant alleles F and S controlled three phenotypes (FF,FS,and SS) with high frequency of Ap^S (0.77) in mares with active ovaries compared to in mare with inactive ovaries(0.50) while, Ap^F showed high frequency (0.50) in animals having inactive ovary compare to cyclic ones (0.23).

Alkaline phosphatase is a dephosphorylating enzyme that is active in many tissues including bone, liver, kidney, intestine, lung, placenta uterus and seminal plasma [18]. It was found that uterine Alkaline phosphatase acitivty markedly increase in infertile mares [11].

In conclusion, It could be concluded that ovarian activity of purebred Arabian mares is controlled by some gene markers Ap^S (0.77), and Es^G (0.64), and this fact must be taken in to consideration in horses used for breeding purposes

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