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Correlations Between Gene Markersof Some Blood Protein Loci and Semen Characteristics in Purebred Arabian Stallions

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Abstract: The current study was designed to find out the possible correlations between gene markers of some blood protein loci and semen characteristics of 18 Purebred Arabian stallions. Serum protein electrophoresis was carried out and gene frequency of genetic loci was estimated: 6 blood protein loci were used as genetic markers Albumin (Al), transferrin (Tf), α-globulin ($f\alpha_2$); Esteas (Es). Alkaline phosphates (Alp) and vitamin D binding protein (Gc). Semen samples were collected from all stallions under investigation and evaluated for different parameters, correlation coefficients were estimated between genetic markers and semen characteristics. Results revealed that mass motility, total motility, individual motility, total sperm and alive sperm were positively correlated ($P<0.001$) with Tf^D, $F\alpha_2^A$, Es^G and Gc^F gene markers, Moreover, the total major abnormalities, total minor abnormalities and sperm abnormalities were positively correlated ($P<0.001$) with Tf^O, $F\alpha_2^B$, Es^H and Gc^S gene markers. It could be concluded that the seminal characteristic of Arabian stallions are genetically controlled and this fact is very important for breeding programs of Arabian horses.

Key words: Arabian stallions · Gene markers · Semen characteristics

INTRODUCTION

The Arabian breed is one of the most influential horse breed in the word. It is worldwide distributed and has been involved in the formation of many other horse breed. In animal breeding,accurate determination of relatedness and efficient control of pidgree registration is of great importance. In the same time, the identification of pidgree information is one of the most difficulties in implementing at breeding programs in horse [1].

Selection of breeding stallions is based on genetics and the desired performance and conformational characteristics. However, with increased use of reproductive technologies and transported semen, stallion fertility is of increasing importance. Even if sub fertile stallions can be identified through conventional semen quality assessments, the ability to select fertile stallions or predict fertility using specific markers is a promising goal [2]. Though a stallion may be fertile over repeated breeding seasons, there is economic value for both the mare and stallion breeder if the stallion has a high first cycle conception rate.

In recent years, research efforts have focused on identifying some reliable markers of fertility at either the genomic or proteomic level. The abundance of four seminal plasma proteins were identified as being negatively related to fertility; these were identified as kallikrein-1E2 (KLK2), clusterin and seminal plasma proteins 1 (SP1) and 2 (SP2). Abundance of cysteine-rich secretory protein 3 (CRISP3) was positively related to first cycle conception rate and may provide a good marker of fertility [3]. Many horse populations are relatively small and an important challenges is to combine the genetic gain in the most valuable trait specific for the breed and at the same time, maintain the genetic variation [4].

Polymorphisms in the gene encoding CRISP3, an abundant seminal plasma protein, have been associated with a reduction in fertility [5]. Because, proteins are involved in various processes to preserve viability of sperm, interactions with the female reproductive tract

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and the process of fertilization [6], they are good candidates for markers of fertility.

The present study was carried out on Purebred Arabian stallions to correlate gene markers of some blood proteins (Albumin (Al), transferrin (Tf), α-globulin ($f\alpha_2$); Esteas (Es). Alkaline phosphates (Alp) and vitamin D binding protein (Gc)) with seminal characteristics.

MATERIALS AND METHODS

Animals: The current study was carried out on 18 Zpurebred 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).

Blood Sampling: After semen collection, blood samples were collected from jugular vein into clean dry sterile and heparinized vacutainer tube. Samples werecentrifugated for 5 min at 3000 r.p.m. Clear plasma were kept at-20°C till biochemical analysis.

Serum Electrophoresis: Electrophoresis patterns of serum proteins was done by polyareylamid gel electrophoresis according to Carlstrom and Johnson [7]. Quantitation of different protein fractions was made using image denistometer (Biorad G700).

Semen Collection and Evaluation: Semen samples were collected from all stallions under investigation using anartificial vagina. Samples were collected (3 times) from each stallion at 15 days apart during spring and summer. Following collection,all semen samples were evaluated according to Dowsett and knott [8]. Ejaculate volume, gell free volume, gell volume, colour score, hydrogen ion concentration, total (normal and abnormal) motility, progressive individual motility, density score, sperm cell concentration, total sperm per ejaculate, total motile sperm, live sperm percentage and sperm cell abnormalitiles were manually determined for each ejaculate.

Gene markers: In the present study, 6 blood protein loci were used as genetic markers Albumin (Al), transferrin (Tf), α-globulin ($f\alpha_2$); Esteas (Es). Alkaline phosphates (Alp) and vitamin D binding protein (Gc).

Quantiativemeasuremnts of Testosterone: The quantitative measurements of testosterone was carried out by using the coat-A-count total testosterone coated tubes radioimmunoassay kit provided by Biochemical laboratories U.S. Washington as described by Abraham [9]. The assay had a senitivity of 0.04 ng / ml with inter and intra assay CV_s both <13 %.

Statistical Analysis: The obtained data were statistically analysis according to Spiegal [10]. Moreovr, correlation coefficients were estimated between genetic markers and semen characteristics.

RESULTS

Table 1 reveals the studied seminal characteristics of Purebred Arabian stallions including volume, color, pH, different forms of motility, density and abnormality. Also, Testosterone plasma level was shown.

Correlation between semen characteristics and gene markers of pure bred Arabian stallion (Table 2). This table reveals that mass motility, total motility, individual motility, total sperm and alive sperm were positively ($P<0.001$) correlated with Tf^D, $F\alpha_2^A$, Es^G and Gc^F gene markers. On the other hands, the total major abnormalities, total minor abnormalities and sperm abnormalities were positively correlated ($P<0.001$) with Tf^O, $F\alpha_2^B$, Es^H and Gc^Sgene markers. Testosterone, Albumin and alkaline phosphatase showed no significant correlation with semen characteristics.

Table1: Semen Characteristics of fertile Purebred Arabian Stallions (Mean ± S.E) n=18.

Semen	Characteristics
Number of ejaculate samples	54
Total volume (ml)	47. 50 ± 1.97
Gell free volume (ml)	42. 55 ± 2.78
Gell (ml)	4. 48 ± 0.28
Color score	2.50 ± 0.13
pH	7. 38 ±0.03
Mass motility	2. 27 ± 0.12
Total motility %	74.50 ± 1.07
Individual progressive motility %	67. 73 ±1.3
Abnormal motility %	6. 27 ± 0.58
Denisty score	2. 02 ± 0.14
Sperm cellconc . ($\times 10^6$/ ml)	310. 64 ±12.2
Total sperm cell per ejaculate ($\times 10^9$/ ml)	14. 62 ± 0.8
Total motile sperm ($\times 10^6$/ ml)	212.8 ± 5.12
Live sperm %	97. 45 ±1.02
Total major sperm abnormalities. %	13. 42 ± 1.63
Total minor sperm abnormalities. %	10.50 ± 0.79
Total sperm abnormalities. (%)	23. 44 ±1.44
Plasma testosterone (ng/ml)	2. 44 ± 0.19

Table 2 :Correlation between gene markers of some blood proten loci and semen characteristics of purebred Arabian stallions

Semen Characteristics	AlF	AlJ	TfD	TfO	Fa2A	Fa2B	EsG	EsH	Apf	ApS	GcF	GcS
Ejaculate volume	..0.27*	0.27*	..0.16	0.16	..0.19	0.19	0.01	..0.1*	0.29	..0.29*	..027*	0.27*
Gell free volume	..026	0.26	0.01	..0.01	..0.1	0.01	0.13	..0.13	0.27	..0.27*	0.08	0.08
Gell volume	0.07	..0.07	..0.29*	0.29*	..0.30*	0.30*	..0.24	0.24	..0.04	..0.04	..0.30*	0.30*
Color score	..0.13	0.13	0.21	..0.21	0.21	..0.21	0.21	..0.21	0.11	..0.11	0.19	..0.19
PH	0.35	..0.35	..0.63***	0.63***	..0.62***	0.62***	..0.61***	0.61***	..0.30	0.30*	..0.56***	0.56***
Mass motility	..0.50***	0.50***	0.84***	..0.84***	0.82***	..082***	0.83***	..0.83***	0.43	..0.43**	0.74***	..0.74***
Total motility	..0.40**	0.41**	0.85***	..0.85***	0.84***	0.84***	0.79***	..0.79***	0.33	..0.33*	0.78***	..0.78***
Individual motility	..0.44**	0.44**	0.89***	..0.89***	0.88***	..0.88***	0.84***	..0.84***	0.37	..0.37**	0.81***	..0.81***
Abnormal motility	0.37**	..0.37**	..0.66***	0.66***	..0.65***	0.65***	..0.64***	0.64***	..0.31	0.31*	..0.59***	0.59***
Density score	..0.08	0.08	0.59***	..0.59***	0.61***	..0.61***	0.46***	..0.46***	0.02	..0.2	0.62***	..0.62***
Sperm cell conc.	..0.25	0.25	0.57***	..0.57***	0.57***	..0.57***	0.53***	..0.53***	0.20	..0.20	0.54***	..0.54***
Total sperm/ejaculate	..0.32	0.32*	0.13	..0.13	0.11	..0.11	0.25	..0.25	0.32	..0.32*	0.03	..0.3
Total motile sperm	..038**	0.38**	0.85***	..0.85***	0.85***	..0.85***	0.78***	..0.78***	0.31	..0.31*	0.79***	..0.79***
Live sperm.	..0.23	0.23	0.83***	..0.83***	0.84***	..0.84***	0.70***	..0.70***	0.16	..0.16	0.82***	..0.82***
Total major sperm abnor.	0.18	..0.18	..0.68***	0.68***	..0.69***	0.69***	..0.57***	0.57***	..0.11	0.11	..0.68***	0.68***
Total minor sperm abnor.	0.24	..0.25	..0.75***	0.75***	..0.76***	0.76***	..0.65***	0.65***	..0.18	0.18	..0.74***	0.74***
Total sperm abnormalities.	0.23	..0.23	..0.77***	0.77***	..0.78***	0.78***	..0.66***	0.66***	..0.16	0.16	..0.77***	0.77***
Plasma testosterone	..0.10	0.10	0.24	..0.24	0.24	..0.24	0.22	..0.22	0.08	..0.088	0.23	..0.23

*P<0.05 significant correlation **P<0.01 highly significant correlation ***P<0.001 very highly significant correlation

*P<0.05 significant correlation **P<0.01 highly significant correlation ***P<0.001 very highly significant correlation

DISCUSSION

The conventional approach to evaluate stallion semen dates back several decades and includes evaluation of spermatozoa concentration, semen volume, spermatozoon morphological characteristics and spermatozoal motility patterns initially and following *in-vitro* storage.

The normal equine spermatozoon differs from that of other large domestic animals in several respects whereas abaxial attachment of the mid piece, asymmetry of the head, small acrosomal volume and small head size distinguish the normal equine spermatozoa. The percentage of normal sperm in the stallion appears lower than that of other domestic animals, with most studies citing between 50 and 60% normal spermatozoa [11-13].

The pH of raw semen should be measured with a pH meter soon after collection. The normal pH of raw semen ranges from 7.2 to 7.7 with a slight increase in pH between the first and second ejaculates [14]. Elevated pH may indicate incomplete ejaculation (pre ejaculatory fluids have a pH of 7.8 to 8.2), urine contamination, inflammation within the genital tract. Samples that are incubated for a period of time after collection will tend to have a lower pH because of the accumulation of metabolic by-products (lactic acid) [14]. Urine contamination of semen (urospermia) can be detected in some ejaculates by a gross change of color or odor while the investigated semen samples showed normal color.

Initial estimation of motility should be made within 5 minutes of collection in both raw and extended semen. Raw stallion semen tends to agglutinate with both head-to-head agglutination and agglutination to the microscope slide. However, estimates of gross motility in raw semen may be useful to identify potential technical problems with the extender or possible adverse effects of the extender on a particular semen sample. Repeatability of estimates of sperm motility are generally better with extended versus raw semen (62 vs. 41%) [15].

Testicular function in the stallion depends on a normal hypothalamic-pituitary-testis axis with a carefully regulated endocrine control, as well as local regulation of cell function by autocrine and paracrine factors within the testis. Roser and others [16] provided evidence that the primary deficit in idiopathic testicular degeneration is related to changes within the testis, which subsequently result in alterations in spermatogenesis and circulating endocrine parameters [15, 17].

Little information is available on AlP activity in the seminal plasma of stallion [18] and this resultis partially in line with our finding because AlP gene marker in present study showed no significant correlation to semen characteristics.

The transaminase activities in semen are good indicator of semen quality because they measure sperm membrane stability [19]. Thus, increasing the percentage of abnormal spermatozoa in ejaculate causes high concentration of transaminase enzyme in the extracellular fluid due to sperm membrane damage and ease of leakage of enzymes from spermatozoa [20,], this physiological

approach could be explained by results of present study as the Tf^{O},$F\alpha_2$ ^{B},Es ^{H} and Gc ^{S} gene markers are responsible for sperm abnormalities as polygenic effect. On the other hand Hinton *et al*. [21]) and Waheed *et.al* [22] reported that glutamyl-transferase (GGT) plays an important role in the protection of spermatozoa from oxidative stress and provides an indicator of a primary testicular and epididymal origin of this enzyme in stallion. Moreover, the present study confirmed that sperm and total motility are controlled by the action of Tf^{D}, $F\alpha_2^{A}$, Es^{G} and Gc^{F} and my be one or more of these gene markers regulate or promote the function of GGT and this result was in line with those obtaind by Taylor *et.al* [23].

The lack of widely available standardized assays for equine gonadotropins has hindered their diagnostic use in subfertile stallions. Endocrine concentrations in stallions must be interpreted in terms of the stallion's age and the season of the year because both of these variables have significant effects on luteinizing hormone (LH) and testosterone concentrations in plasma [24]. The current study revealed normal values of testosterone While semen analysis performed in this manner does have predictive value, the DNA strand breaks can be associated with a myriad of factors, including diopathic apoptosis, oxidative stress, heat stress, radiation injury, or protamine deficiency [25-27] and may involve double-stranded or single-stranded DNA fragmentation, or oxidized nucleosides [28]. Such lesions could create genetically defective spermatozoa, leading to germ-line mutations. Interestingly, spermatozoa affected by such damage may appear to be normal, based on laboratory parameters such as spermatozoa motility and membrane integrity, but may induce post fertilization embryonic failure [29], so it is logical that DNA damage might not be expressed until mitosis occurs at the time of spermatozoon-oocyte fusion. This becomes quiteimportant clinically where increasing the insemination number will not increase pregnancy rate.

The ability to select these fertile stallions or predict *in vivo* fertility using biomarkers is a promising goal. In recent years, research efforts have focused on identifying reliable biomarkers of fertility using genomic and protomic levels.

In conclusion, the present study revelaed significant correlation between good semen quality associated with high fertility and the blood protein Tf D, $F\alpha_2^{A}$, Es^{G} and Gc^{F} gene markers. These are newly suggested good candidates for serum proteomic and genomic markers of fertility in Arabian stallions.

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