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Correlation Between Semen Characteristics and Some Gene Markers in Purebred Arabian Stallions

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Abstract: The present study was carried out on 28 Arabian stallions at Al Zahraa stud, Ain Shams, Cairo, Egypt. According to breeding history, sexual behavior and clinical examination, stallions were divided into 2 groups;fertile (n=18) and the infertile (n=10) groups. Semen samples were collected using artificial vagina and semen characteristics were evaluated. After semen collection, blood samples were taken and plasma was separated after centrifugation, to measure the testosterone(RIA) level and, perform immunogenetic analysis. Results showed that fertile stallions were characterized by significantly ($p<0.05$) increase, in total (normal and abnormal) motility, sperm cell concentration and incidence of live spermatozoa and significantly ($p<0.05$) decrease in total sperm abnormalities. Meanwhile, infertile stallions revealed. significantly ($p<0.05$) increase in total sperm, abnormalities and decrease of sperm cell concentration, total motility and live spermatozoa. Plasma testosterone level (ng/ml) averaged 2.44±0.23 and 1.97±0,25 for fertile and infertile stallions, respectively. Correlation coefficient showed that motility (individual and total), total motile sperm and alive sperm were positively correlated ($p<0.01$) with Tf^D, Es^G and Gc^F gene markers (r = 0.84, 0.83 and 0.74, respectively) while these parameters were negatively correlated with Tf^O, Gc^S gene markers (r = - 0.84. and -0.74, respectively). It was concluded that semen characteristics in Arabian stallions are controlled by some gene markers which, could be used for a future prediction of fertility in selection programs.

Key words: Semen · Fertility · Arabian stallions · Gene markers

INTRODUCTION

Evaluation of the breeding stallion for reduced fertility has relied on physical examination of the reproductive system as well as evaluation of sperm number, motility and morphology. Additionally there are some other diagnostic methods which become available to facilitate reproductive evaluation of the breeding stallion, such as ultrasonography and sperm function assays [1].

Recent studies have shown that male fertility does not only depend on the absolute number of viable, motile, morphologically normal sperm that can be inseminated in a female. Rather, a more important parameter appears to be the functional competence of sperm cells – since this cannot be evaluated using a single variable. Researchers have proposed that semen samples should be subjected to multi- parametric analysis [2 - 4]. Correlation between fertility and genetic constitution of stallions had been discussed [4-8]. There are more than 200 known genes which are involved in the production of the fertile sperm cell. Great progress has been made in the understanding of genetic aberrations that lead to male infertility [9].

Semen and seminal plasma as well as testosterone level of Arabian stallion have been investigated in relation to breed difference [10] as well as quantitative and qualitative characteristics of semen were studied in stallion by Ball [1], Oba *et al.* [11], Lendeberg *et al.* [12], Hafez and Hafez [13], Abdel-Rahmman [14], Lefrapper *et al.* [15] and Brito *et al.* [16].

The present investigation aimed to study the correlation between semen characteristics and gene markers of purebred Arabian stallions reaised at Egypt.

MATERIALS AND METHODS

Animals: The present study was carried out on 18 purebred Arabian stallions (aged 5-6 year). Animals were kept at Al-Zahraa Stud Ain-Shams, Cairo, Egypt. Animals 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 Darrawa).

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Special care for diseases control including regular application of anti parasitic drugs (against external and internal parasites) was taken in consideration.

Experimental Design: According to the breeding history, sexual behavior, clinical examination and pregnancy rates[17], stallions were divided in to 2 groups:

- Fertile stallions (n=18) had no history of any breeding problem with pregnancy rate up to 70% and with healthy normal genitalia.
- Inferile stallions (n=10) with different testicular and scrotal affections (unilateral cryptorchidism, testicular degeneration and orchitis) with pregnancy rate <10%.

Semen Collection and Evaluation: Semen samples were collected from all stallions under investigation using an artificial vagina (Missouri). Samples were collected (3 times) from each stallion at 15 days apart during spring and summer. After that, all semen samples were evaluated according to Dowsett and Knott [18]. Ejaculate volume, gel free volume, gel 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 abnormalities were manually determined for each ejaculate.

Blood Sampling: After semen collection, blood samples were collected from jugular vein into clean dry sterile and heparinized vacutainer tube. Samples were centrifugated for 5 min at 3000 r.p.m. Clear plasma were aspirated by Pasteur pipettes and transferred into dry sterile labeled stoppered Eppendorff vials and kept at - 20°C till biochemical analysis.

Analysis

Quantitative measurements 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, Washington,USA, as described by Blodow *et al.* [19]. The assay had a sensitivity of 0.04 ng/ml with inter and intra assay CVs both < 13%.

Protein Electrophoresis: Electrophoresis patterns of plasma proteins was done by polyacrylamid gel electrophoresis according to Carlstrom and Johnson [20]. Quantitation of different protein fractions was made using image denistometer (Biorad G 700).

Statistical Analysis: The obtained data were statistically analysis according to Spiegal [21]. Moreover, correlation coefficients were estimated between genetic markers and different semen characteristics.

RESULTS

The obtained results are presented in tables 1 and 2. Table1 reveals the semen characteristics and plasma testosterone level in both fertile and infertile stallions. The largest total ejaculate volume (ml) was found in the fertile (47±1.9) as compared with the infertile (45.17±3.52) stallions for infertile). Meanwhile, the gell free volume and gell volume did not vary among fertile and infertile stallions. The mass motility, percent of total and progressive individual motility, sperm cell concentration, total sperm per ejaculate, total motile sperm and live sperm percent significantly increased, while, the hydrogen ion concentration and percent of abnormal motility and different types of sperm cell abnormalities significantly decreased in fertile (Particularly those with high fertility) than infertile stallions. Plasma testosterone levels revealed no significant changes as regard to fertility (Table 1).

Table 1: Semen characteristics and plasma testosterone level in fertile and infertile stallions (Mean±SE)

Parameters	Fertile stallions	Infertile stallions
(A) Semen Characteristics:		
Number of animals	18	10
Number of ejaculate samples	24	18
Total volume (ml)	47 ± 1.9^{b}	45.17 ± 3.52^{ab}
Gell free volume (ml)	42.6 ± 2.7^{a}	39.83 ± 3.23^{a}
Cell (ml)	4.4 ± 0.28^{a}	5.22 ± 0.47^{a}
Color score	2.50 ± 0.13^{a}	2.19 ± 0.19^{a}
PH	7.4 ± 0.03^{b}	7.68 ± 0.05^{a}
Mass motility	2.27 ± 0.12^{a}	0.83 ± 0.11^{c}
Total motility (%)	75.00 ± 1.15^{a}	53.06 ± 2.16^{c}
Individual motility (%)	68.23 ± 1.3^{a}	38.61 ± 2.54^{c}
Abnormal motility (%)	6.77 ± 0.58^{b}	14.44 ± 1.46^{a}
Density score	2.02 ± 0.14^{a}	1.33 ± 0.1^{c}
Sperm cell conc..($xl0^{6}$/ml)	310.64 ± 12.77^{a}	218.22 ± 19.91^{b}
Total sperm per ejac. ($x10^{9}$/ml)	14.62 ± 12.77^{a}	10.45 ± 1.44^{b}
Total motile sperm ($xI0^{6}$/ml)	212.81 ± 10.12^{a}	83.87 ± 7.56^{c}
Live sperm (%)	79.46 ± 1.02^{a}	$63.17+ 1.84^{c}$
Total major sperm abn. (%)	13.42 ± 0.81^{c}	19.89 ± 1.11^{a}
Total minor sperm abn. (%)	10.5 ± 0.79^{c}	18.22 ± 0.87^{a}
Total sperm abn.(%)	23.91 ± 1.44^{c}	38.11 ± 1.66^{a}
(B) Plasma testosterone (ng/mt)	2.44 ± 0.23^{a}	1.97 ± 0.25^{a}

Means with different superscripts in each category are significantly different from each other at least at ($P < 0.05$).

Table 2: Correlation between genes frequency and semen characteristics of Arabian stallions

Semen characteristics	Al^F	Al^J	Tf^D	Tf^O	Es^C	Es^H	Ap^F	Ap^S	Gc^F	Gc^S
Ejaculate volume	-0.27*	0.27*	-0.16	0.16	0.01	-0.01*	0.29	-0.29*	-0.27*	0.27*
Gel) free volume	-0.26	0.26	0.01	-0.01	0.13	-0.13	0.27	-0.27*	-0.08	0.08
Cell volume	0.07	-0.07	-0.29*	0.29*	-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	0.11	-0.11	0.19	-0.19
PH	0.35	-0.35*	-0.63***	0.63***	-0.61***	0.61***	-0.30	0.30*	-0.56***	0.56***
Mass motility	-0.50***	0.50***	0.84***	-0.84***	0.83***	-0.83***	0.43	-0.43***	0.74***	-0.74***
Total motility	-0.40**	0.41***	0.85***	-0.85***	0.79***	-0.79***	0.33	-0.33*	0.78***	-0.78***
Individual motility	-0.44***	0.44***	0.89***	-0.89***	0.84***	-0.84***	0.37	-0.37***	0.81***	-0.81***
Abnormal motility	0.37**	-0.37***	-0.66***	0.66***	-0.64***	0.64***	-0.31	0.31*	-0.59***	0.59***
Density score	-0.08	0.08	0.59***	-0.59***	0.46***	-0.46***	0.02	-0.02	0.62***	-0.62***
Sperm cell concentration	-0.25	0.25	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	0.25	-0.25	0.32	-0.32*	0.03	-0.03
Total motile sperm	-038**	0.38**	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***	0.70***	-0.70***	0.16	-0.16	0.82***	-0.82***
Total major sperm abnormalities	0.18	-0.18	-0.68***	0.68***	-0.57***	0.57***	-0.11	0.11	-0.68***	0.68***
Total minor sperm abnormalities	0.24	-0.25	-0.75***	0.75***	-0.65***	0.65***	-0.18	0.18	-0.74***	0.74***
Total sperm abnormalities	0.23	-0.23	-0.77***	0.77***	-0.66***	0.66***	-0.16	0.16	-0.77***	0.77***
Plasma testosterone	-0.10	0.10	0.24	-0.24	0.22	-0.22	0.08	-0.08	0.23	-0.23

*P<0.05 **P<0.01 *** P<0.001

Table (2) showed correlations between immunogenetic markers and semen characters. Results of correlation test showed highly significant positive correlation (P< 0.001) between motility, live sperm and total motile sperm with Tf^D, Es^G and Gc^F gene markers, while these parameters were negatively correlated with Tf^O and Gc^S gene markers. Meanwhile total sperm cell abnormalities was positively correlated with Tf^O and Gc^S gene markers.

DISCUSSION

Stallion Fertility is an economically important trait because the use of artificial insemination is increasing in the horse industry and superior sires are used more intensely. Molecular genetic markers may be useful as early indicator for a stallion fertility and genetic improvement programs [22, 23]. Genetic markers in candidate genes, such as CR/ SP3 SPATA1 and INHBA, in breeding stallions have been associated with pregnancy rate per oestrus in mares [6]. They also reported candidate autosomal X and Y genes for stallion fertility, including genes encoding hormones and their receptors of the hypothalamic- pituitary axis, proteins of the seminal plasma, protein involved in spermatozoa – ovum binding and genes influencing sexual development, as well as Y- specific genes.

In the present study, 5 blood protein loci were used as genetic markers for investigating the immunogenetic constituents of Arabian stallions in relation to fertility status [7]. Moreover, correlations were estimated between genetic markers and semen characteristics in order to predict the future fertility at younger ages depending upon gene markers associated with high fertility.

Concerning semen characteristics, the results of the present study indicated that high fertile stallions were specially characterized by high incidence of motile sperm and sperm cell concentration and low incidence of sperm abnormality. These findings were in line with those reported by AK-J *et al.* [9], Vineyard *et al.* [24], Alv*arenga et al.* [25], Phetudomsinsuk [27] and Dogan *et al.* [28] especially for ejaculate volume, sperm motility and alive spermatozoa. Meanwhile, dissimilar results were recorded by Terry *et al.* [5] and Hammes *et al.* [26] for thoroughbred stallion and Kosiniok *et al.* [29] for both wielkopolski and Malopolski horses and the condition may be due to the lowest genetic similarity between both breeds [5, 9, 30, 31]. However, variation in semen quality among breeds as indicator for fertility of stallion is common because there is no single test that can serve as an absolute indicator of fertility for stallions [1, 2, 32].

In the current investigation, plasma testosterone levels (ng/ml) averaged 2.44±0.23 ng/ml in fertile and 1.97±0.25 ng/ml in infertile stallions. These results were more or less in accordance with those reported by Abdel Rahmman [13] and Mckinnon and Voss [33] in the same breed. However, higher testosterone levels were reported by Abu Nawwara [34] with average of 3.19±0.12 and 2.91±0.21 ng/ml for fertile and infertile Arabian horses.

Variations in testosterone levels are attributed to seasonal effects [33]. Moreover, it was reported that sexual behavior in horses is mainly affected by plasma estradiol 17 β and Cortisol levels rather than testosterone level [13].

The correlation between enzymatic activity and semen characterstics had been established, Turner and McDonnell [35] reported that a significant correlation between alkaline phosphates (ALP) with total sperm number. Moreover, Dogan *et al.* [26] recorded that the majority of ALP in bulls originates from seminal vesicles and to a lesser extent from the tests and epididymides, but little information is available on ALP activity in seminal plasma of stallions.

On the same time Pesch *et al.* [36] reported the correlation between lactate dehydrogenase LDH and motility, progressive motility and living sperm which may indicates that extra cellular LDH ensures metabolism of spermatozoa. In addition Ciereszko *et al.* [37] reported a high correlation between ALP activity released by spermatozoa and semen quality when sperm cells were subjected to minimal and maximal stress. Likewise, ALP, AST and LDH are essential for metabolic processes which provide energy for survival motility and fertility of spermatozoa. These correlations need more scientific efforts to detect specific gene markers responsible for each criteria of semen characteristics.

In present study results of correlation coefficient showed a highly significant positive correlation between motility, live sperm and total motile sperm with Tf^{D}, Es^{G} and Gc^{F} gene markers, while, these parameter were negatively correlated with Tf^{O} and Gc^{S} gene markers. Meanwhile, total sperm cell abnormalities were positively correlated with Tf^{O} gene marker. In this respect Zaabal *et al.* [38] reported a correlation between immumogenetic markers of serum proteins and seminal plasma of cattle and buffalo bulls and they found a correlation between $S\alpha_2^{B}$ marker in seminal plasma and Pr^{B} marker of serum protein in fertile buffalo. In stallions correlation between semen characteristics and gene markers of blood proteins and enzymes is still limited argument.

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