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STUDIES ON SEMEN CHARACTERISTICS OF FERTILE AND INFERTILE PUREBRED ARABIAN STALLIONS IN RELATION TO IMMUNOGENETIC MARKERS

(With 3 Tables and 1 Figure)

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دراسات على خصائص السائل المنوي في الخيول العربية الأصيلة الخصوبة والغير خصوبة وعلاقتها ببعض دلالات المناعة الوراثية

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أجريت هذه الدراسة على ١٨ جوادا عربيا اصيلا بمزرعة الزهراء – عين شمس القاهرة في الفترة من ١٩٩٨-٢٠٠١ بهدف دراسة خصائص السائل المنوي وعلاقة هذه الخصائص بالمكونات المناعية الوراثية لهذه الخيول وتم تقسيم هذه الخيول إلى ثلاثة مجموعات: المجموعة الأولى عالية الخصوبة (٨ حصان) والثانية منخفضة الخصوبة (٤ حصان) والثالثة ذات خصوبة منخفضة جدا (٦ حصان). تم تجميع عينات السائل المنوي باستخدام المهبل الصناعي وتم تقييمها من حيث كمية السائل المنوي والحركة الفردية والجماعية للحيامن ونسبة تركيز الحيامن وكذلك نسبة الحيامن الحية والغير طبيعية . كما أخذت عينات الدم وفصلت البلازما لقياس مستوى التستوستيرون وإجراء تحاليل المناعة الوراثية. وقد أشارت النتائج إلى أن خيول المجموعة الأولى تتميز بزيادة معنوية في الحركة الفردية والجماعية للحيامن وتركيز الحيامن وكذلك نسبة الحيامن الحية وانخفضت معنويا نسبة الحيامن غير الطبيعية وكان مستوى التستوستيرون في البلازما ٢,٥٠ ± ٠,١٨ نانوجرام/مل. أما خيول المجموعة الثانية فكانت نتائج تقييم السائل المنوي اقل من الخيول عالية الخصوبة أما خيول المجموعة الثالثة فقد عبرت نتائجها عن زيادة معنوية في نسبة الحيامن غير الطبيعية وكذلك نسبة الحيامن الحية في حين انخفضت معنويا نسبة حركة الحيامن وتركيزها. بالنسبة لتحاليل المناعة الوراثية فقد أكدت النتائج وجود ارتباط بين بعض الاليلات الوراثية وبين الخصوبة حيث تميزت خيول المجموعة الأولى بسيادة الجينات الوراثية التالية Gc^{F} Tf^{D} , $F\propto 2^{A}$, Es^{G} and Es^{H}, Gc and Tf^{O} في حين تميزت خيول المجموعة الثانية بسيادة الجينات التالية Ap^{F} و Es^{G} وفي خيول المجموعة الثالثة سادت الجينات

Es^S مع سيادة كبيرة تكاد تكون مطلقة للجين Es^H حيث كان تكراره (٠,٩١٦) وهو ما قد يرجح مسئوليته عن انعدام الخصوبة في الخيول. وإشارت اختبارات الارتباط إلى ارتباط معنوي بين النسبة المئوية لحركة الحيامن (جماعية وفردية) ونسبة الحيامن الحية وكذلك عدد الحيامن المتحركة والجينات التالية Tf^D , $F\alpha2^A$, Es^G and Gc^F بينما أظهرت النتائج ارتباط معنوي سلبي بين القيم السابقة والجينات Tf^O , $F\alpha2^B$ and , Gc^S . وخلص الباحثون إلى ان السائل المنوي في الخيول العربية تتحكم في خصائصه بعض الجينات الوراثية التي يمكن استخدامها في التنبؤ المبكر للخصوبة .

SUMMARY

The present study was carried out on 18 purebred Arabian stallions during a period of 4 years (1998 –2001). Animals were kept at Al Zahraa Stud, Ain Shams, Cairo-Egypt. According to breeding history, sexual behavior and clinical examination, stallions were divided into 3 groups: fertile (n=8), subfertile (n=4) and infertile (n=6). Semen samples were collected using artificial vagina and semen characteristic were evaluated. After semen collection, blood samples were taken, and plasma was separated after centrifugation, to measure the testosterone 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 2.50 ± 0.18 and 1.97 ± 0.25 for fertile and infertile stallions respectively. Concerning, immunogenetic analysis, results revealed that fertile stallions showed predominance of Tf^D, $F\alpha2^A$, Es^G and Gc^F gene markers while, sub fertile animals showed high frequency of Ap^F and Gc^S gene markers. Infertile stallions revealed high frequency of Es^H, Gc^S and Tf^O with apparently absolute predominance of Gc^S gene marker (0.916). Correlation coefficient showed that motility (individual and total), total motile sperm and alive sperm were positively highly significantly ($p<0.001$) correlated with Tf^D, $F\alpha2^A$, Es^G and Gc^F gene markers ($r=0.84$, 0.82, 0.83 and 0.74 respectively) while these parameters were negatively correlated with Tf^O, $F\alpha2^B$, Gc^S gene markers ($r=-0.84$, -0.82, and -0.74 respectively). It is concluded that semen characteristics in Arabian stallions are

controlled by some gene markers which could be used for a future prediction of fertility.

Key words: Semen, fertility, Arabian stallions, immunogenetic markers.

INTRODUCTION

The ability of the stallion to breed mares and get them pregnant with subsequent birth of alive foal is a fundamental criterion to any reproductive programme in horses (Hammes *et al.*, 1996).

Sexual behavioral parameters of Arabian stallion such as latency to erection, latency to mount, latency to ejaculation and number of mounts per ejaculate were affected by month of the year, age, season as well as individual variations (Vinaya *et al.*, 1999 and Adel – Rahmman 2001).

Semen and seminal plasma as well as testosterone level of Arabian stallion have been investigated in relation to breed difference (AK-K *et al.*, 1994) as well as quantitative and qualitative characteristics of semen were studied in stallion by Oba *et al.*, 1993; Lendeberg *et al.*, 1999; Hafez and Hafez 2000 and Abdel- Rahmman, 2001.

The highly fertile stallion achieved 75-100% pregnancy rates per-cycle. Such stallion has uniformly certain characteristics including large normal testes, good mating technique, excellent sperm motility and longevity, and few morphologically abnormal sperm (Hurtgen, 1997).

Infertility due to genetic causes has been discussed by Tainturier *et al.* (1995) in stallions, and Larsen *et al.* (2001) in mares. This phenomenon is common but frequently not recognized by farm manager or mare owners. Investigations on the relationship between genetic markers and variation in quantitative traits are of interest from perspectives of both theoretical quantitative genetics and practical animal breeding, and this subject was studied through analysis of the reproductive performance of Arabian stallion (Pikula *et al.*, 2001).

Immunogenetic markers associated with fertility status of Arabian stallion had been established by many authors (Vega *et al.*, 1998; Gralak *et al.*, 2000; Nogaj and Nogaj 2000 and Pikula *et al.*, 2001).

The present study aimed to evaluate the semen characteristics of Arabian stallions with special reference to the immunogenetic markers.

MATERIALS and METHODS

(I) Animals:

The present study was carried out on 18 purebred Arabian stallions (aged 5-6 year) during a period of 4 years (1998- 2001). 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). Special care for diseases control including regular application of anti parasitic drugs (against external and internal parasites) was taken in consideration.

(II) Experimental design:

During the experimental period (4year), stallions were closely followed up and according to the breeding history, sexual behavior, clinical examination and pregnancy rates (Kenney *et al.*, 1991), the present stallions were divided into three groups:

Group (1): fertile stallions (n=8), which had no history of any breeding problems with pregnancy, rate up to 70% and with apparently healthy normal genitalia.

Group (2): subfertile stallions (n=4) were referred with a history of a breeding problem that was subsequently determined not to be attributable to the mares or infectious diseases. With pregnancy rate up to 30% and with healthy normal genitalia.

Group (3): infertile stallions (n=6) 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. 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 (1996). 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 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:

(A) 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 U.S. Washington as described by Blodow *et al.* (1988). The assay had a sensitivity of 0.04 ng/ml with inter and intra assay CVs both < 13%.

(B) Protein electrophoresis: Electrophoresis patterns of plasma proteins was done by polyacrylamid gel electrophoresis according to Carlstrom and Johnson (1983). Quantitation of different protein fractions was made using image denistometer (Biorad G 700).

Genetic parameters:

(A) Immunogenetic markers: In the present study, 6 blood protein loci were used as immunogenetic markers Albumin (Al), transferrin (Tf), ∝ - globulin (f∝2); Estras (Es). Alkaline phosphates (AP) and vitamin D binding protein (Gc).

(B) Heterozygosity (SH): The heterozygosity at the electrophoretic loci that are all codominant unequivocally determined by counting the number of heterozygous loci for each animal (Anderson and Davies 1993).

(C) Gene Frequency: Gene frequencies were counted as the expected Hard Weinberg proportion of heterozygous genotypes in that particular phenotype class (Merkoreva, 1977 and Anderson and Davies 1993).

(E) Statistical analysis: The obtained data were statistically analysis according to **Spiegal (1988).** Moreover, correlation coefficients were estimated between genetic markers and semen characteristics.

RESULTS

The obtained results are presented in Tables (1-3). Table (1) reveals the semen characteristics and plasma testosterone level in both fertile and infertile stallions. The largest total ejaculate volume was

found in sub fertile males, followed by the infertile ones and those with high fertility. 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 significantely decreased in fertile (Particularly those with high fertility) than infertile stallions. Plasma testosterone levels revealed no significant changes as regard to fertility (Table 1).

Electrophoretic patterns of plasma proteins are shown in Fig. (1). However, Table (2) reveals the genotyping and gene frequencies of 6 blood protein loci in relation to fertility in Arabian stallions. Results indicated that high fertile stallions were characterized by high frequency of Tf^{D}, $F\alpha 2^{A}$, Es^{G}, and Gc^{F} gene markers, while sub fertile ones showed high frequency of Ap^{F} and Gc^{S} gene marker. Infertile stallions distinguished by high frequency of Es^{H}, Gc^{S} and Tf^{O} with predominance of Es^{H} (0.916).

Table (3) 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}, $F\alpha\ 2^{A}$, Es^{G} and Gc^{F} gene markers, while these parameters were negatively correlated with Tf^{O}, $F\alpha\ 2^{B}$ and Gc^{S} gene markers. Meanwhile total sperm cell abnormalities was positively correlated with Tf^{O} and $F\alpha\ 2^{B}$ gene markers.

Coefficient of heterozygosity in studied stallions recorded 0.089 for fertile, 0.077 in sub fertile and 0.063 for infertile ones.

DISCUSSION

The close relationship between reproductive biology and genetic improvement in farm animals has been recognized for a long time. A high reproductive performance enables a reduction in generation length and / or an increase in selection differential (Hafez and Hafez, 2000).

Immunogenetic markers that associated with the fertility of Arabian stallions have been established by many authors (Vega *et al.*, 1998; Gralak *et al.*, 2000, Nogaj and Nogaj 2000; Pikula *et al.*, 2001 and Larsen *et al.*, 2001).

In the present study, 6 blood protein loci were used as genetic markers for investigating the immunogenetic constituents of Arabian

horses in relation to fertility status. 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-K *et al.* (1994) Vineyard *et al.*, (1999) and Altadena et al, (2000) especially for ejaculate volume, sperm motility and alive spermatozoa. Meanwhile, dissimilar results were recorded by Hammes *et al.* (1996) for thoroughbred stallion and the condition may be due to the lowest genetic similarity between both breeds (Han, 1995 and Cunningham, 1991). 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 (Peter *et al.*, 1991).

In the current investigation, plasma testosterone levels (ng/ml) averaged 2.50 ± 0.18 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 Mckinnon and Voss (1993) and Abdel Rahmman (2001) in the same breed. However, higher testosterone levels were reported by Abu Nawwara (2000) 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(Mckinnon and Voss ,1993). Moreover, it was reported that sexual behavior in horses is mainly affected by plasma estradiol 17 β and cortisol levels rather than testosterone level (Abdel Rahmman, 2001).

From the Immunogenetic point of view, constitution of genetic markers of Arabian stallions allover the world has been studied (Tomaszewska, 1994; Pikula *et al.*, 1997 and 20001 and Gronet, *et al.*, 2000). Also, relationships between specific genetic markers and fertility status are argument for fertility (Langlois 1999; Niemczewski and Zurkowski 2000 and Larsen *et al.*, 2001) and for infertility (Vijh *et al.*, 1990 and Hellander *et al.*, 1991).

In this study, it was found that fertile stallions that have good semen quality showed predominance of TF^D, $F\alpha2^A$ Es^G and Gc^F gene makers. These results (specially for Gc^F gene marker) was similar to those recorded by Carvalho *et al.* (1998), Niemczewski and Zurkowski (2000), Kuryl (1997), Gronet *et al.* (2000) and Sasimowski *et al.* (2000)

and confirmed the finding of Bougler (1999) who reported that Gc locus plays very important role in stallion fertility. In this respect, Cho-Giljae *et al.* (2000) reported that fertile Cheju horses in Korea, is characterized by high frequency of Al, Gc^F gene markers and complete dominance of Ap^S marker. In this regard Ap^S, marker in high fertile stallion in the present study recorded high frequency but not completely dominant. On the other hand Sasimowski et al (2000) revealed high frequency of Gc^S, Tf^D and Tf^R markers in fertile felin ponies population in Poland. while Smugala *et al.* (1999) reported a relationship between Es and TF loci and fertility in purebred Arabian horses. However Rodriguez *et al.* (1990) used transferrin allele as a genetic marker for fertility in Spanish Thoroughbred horses.

Infertile stallions in the present study were characterized by high frequency of Es^H, Gc^S and Tf^O gene markers with apparently complete predominance of Es^H as its frequency was (0.916). These findings were partially in agreement with those reported by Hellander *et al.* (1991) and Kenney and Love (1994). The remarkable result of infertile stallions was the predominance of homozygotic genotypes in most studied loci. It can be suggested that infertility as a reproductive trait is governed by dominant homozygotic genes. (Hafez and Hafez 2000).

In present study results of correlation coefficient showed a highly significant positive correlation between motility, live sperm and total motile sperm with Tf^D, $F\alpha\ 2^A$, Es^G and Gc^F gene markers, while, these parameter were negatively correlated with Tf^O, $F\alpha\ 2^B$ and Gc^S gene markers. Meanwhile, total sperm cell abnormalities were positively correlated with Tf^O and $F\alpha\ 2^B$ gene markers. In this respect Zaabal *et al.*(1996) 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 immunogenetic markers is still limited argument.

Concerning coefficient of heterozygosity in the present study, results revealed that this coefficient was 0.089, 0.077 and 0.063 for fertile, sub fertile and infertile stallions respectively. These results were in agreement with the findings recorded by (Reklewski *et al.*, 1997) for Polish Arabian horse, but disagree with results of Pikula *et al.* (1997) and Cho- Gijlae (2000) for Cheju horses and the condition may be mainly attributed to genetic variation among breeds.

It could be concluded that fertility of Arabian stallions controlled by some gene markers could be taken in to consideration in horses used for breeding purposes, specially those which are closely related to high fertility.

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

Parameters	*Fertile stallions*		*Infertile stallions*
	High fertile	*Sub fertile*	
(A)Semen Characteristics:			
Number of animals	8	4	6
Number of ejaculate samples	24	12	18
Total volume (ml)	43.13 ± 1.50 ^{b}	51.00 ± 2.45 ^{a}	45.17 ± 3.52 ^{ab}
Gell free volume (ml)	38.88 ± 3.11^{a}	46.33 ± 2.46 ^{a}	39.83 ± 3.23^{a}
Gell (ml)	4.21 ± 0.26 ^{a}	4.67 ± 0.30 ^{a}	5.22 ± 0.47 ^{a}
Color score	2.50 ± 0.12 ^{a}	2.50 ± 0.14 ^{a}	2.19 ± 0.19 ^{a}
PH	7.39 ± 0.03 ^{b}	7.41 ± 0.04 ^{b}	7.68 ± 0.05 ^{a}
Mass motility	2.54 ± 0.10 ^{a}	2.00 ± 0.14 ^{b}	0.83 ± 0.11 ^{c}
Total motility (%)	77.08 ± 1.06 ^{a}	72.92 ± 1.50 ^{b}	53.06 ± 2.16 ^{c}
Individual motility (%)	70.63 ± 0.85 ^{a}	65.83 ± 1.75 ^{b}	38.61 ± 2.54^{c}
Abnormal motility (%)	6.46 ± 0.46 ^{b}	7.08 ± 0.71 ^{b}	14.44 ± 1.46 ^{a}
Density score	2.29 ± 0.11 ^{a}	1.75 ± 0.17 ^{b}	1.33 ± 0.11 ^{b}
Sperm cell conc. (x10^{6}/ml)	321.71 ± 11.54^{a}	299.58 ± 14.01 ^{a}	218.22 ± 19.91^{b}
Total sperm per ejac. (x10^{6}/ml)	13.93 ± 0.62 ^{a}	15.31 ± 0.98 ^{a}	10.45 ± 1.44^{b}
Total motile sperm (x10^{6}/ml)	227.74 ± 9.13 ^{a}	197.88 ± 11.11 ^{b}	83.87 ± 7.56 ^{c}
Live sperm (%)	83.50 ± 0.95 ^{a}	75.42 ± 1.10 ^{b}	63.17 ± 1.84 ^{c}
Total major sperm abn. (%)	11.67 ± 0.56 ^{c}	15.17 ± 1.07 ^{b}	19.89 ± 1.11 ^{a}
Total minor sperm abn. (%)	8.83 ± 0.75 ^{c}	12.17 ± 0.84 ^{b}	18.22 ± 0.87 ^{a}
Total sperm abn.(%)	20.50 ± 1.10 ^{c}	27.33 ± 1.78 ^{b}	38.11 ± 1.66 ^{a}
(B) Plasma testosterone (ng / ml)	2.50 ± 0.18 ^{a}	2.38 ± 0.28 ^{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): Genotyping and gene frequencies of blood protein loci in relation to fertility in Arabian stallions

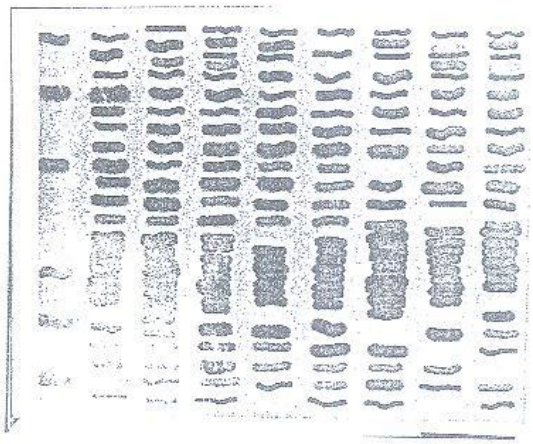
	Albumin	*Transferrin*	*Alpha-globulin*	*Estrase*	*Alkaline phosphatas*	*Vitamin D binding protein*
fertile N=8	FF* 3** (2.5)*** FJ 3 (4.0) JJ 2 (1.5) Gene frequency $Al^F = 0.565$ $Al^J = 0.435$ $X^2 = 0.55$	DD 5 (4.5) DO 2 (3.0) OO 1 (0.5) Gene frequency $TF^D = 0.75$ $TF^O = 0.25$ $X^2 = 0.85$	AA 6 (5.3) AB 1 (2.4) BB 1 (0.3) Gene frequency $F\alpha_2{}^A = 0.812$ $F\alpha_2{}^B = 0.187$ $X^2 = 2.5$	GG 4 (3.7) GH 3 (3.5) HH 1 (0.8) Gene frequency $Es^G = 0.687$ $Es^H = 0.313$ $X^2 = 0.12$	FF 2 (1.1) FS 2 (3.8) SS 4 (3.1) Gene frequency $AP^F = 0.375$ $AP^S = 0.624$ $X^2 = 1.8$	FF 5 (3.7) FS 1 (3.5) SS 2 (0.8) Gene frequency $GC^F = 0.687$ $GC^S = 0.312$ $X^2 = 4$
Sub fertile N=4	FF 1 (1) FJ 3 (2) JJ 1 (1) Gene frequency $Al^F = 0.5$ $Al^J = 0.5$ $X^2 = 0.0$	DD 2 (1.5) DO 1 (2.0) OO 1 (0.5) Gene frequency $TF^D = 0.625$ $TF^O = 0.374$ $X^2 = 1.2$	AA 1 (1.5) AB 1 (2) BB 2 (0.5) Gene frequency $F\alpha_2{}^A = 0.624$ $F\alpha_2{}^B = 0.375$ $X^2 = 1.2$	GG 3 (3) GH 1 (1) HH 0.0 (0.0) Gene frequency $Es^G = 0.875$ $Es^H = 0.125$ $X^2 = 0.0$	FF 1 (1.5) FS 3 (2.0) SS 0.0 (0.5) Gene frequency $AP^F = 0.625$ $AP^S = 0.374$ $X^2 = 0.7$	FF 1 (0.5) FS 1 (2) SS 2 (1.5) Gene frequency $GC^F = 0.375$ $GC^S = 0.625$ $X^2 = 1.2$
Infertile N=6	FF 3 (2) FJ 1 (3) JJ 2 (1) Gene frequency $Al^F = 0.583$ $Al^J = 0.416$ $X^2 = 2.8$	DD 1 (0.3)) DO 1 (2.4) OO 4 (3.3) Gene frequency $TF^D = 0.25$ $TF^O = 0.75$ $X^2 = 2.4$	AA 0.0 (0.3) AB 3 (2.4) BB 3 (3.3) Gene frequency $F\alpha_2{}^A = 0.25$ $F\alpha_2{}^B = 0.75$ $X^2 = 0.13$	GG 0.0 (0.0) GH 1 (1) HB 5 (5) Gene frequency $Es^G = 0.083$ $Es^H = 0.916$ $X^2 = 0.0$	FF 1 (0.6) FS 2 (2.8) SS 3 (2.6) Gene frequency $AP^F = 0.333$ $AP^S = 0.666$ $X^2 = 0.56$	FF 1 (0.2) FS 0.0 (1.6) SS 5 (4.2) Gene frequency $GC^F = 0.166$ $GC^S = 0.833$ $X^2 = 3.3$

x genotypes *xx observed no of genotype* *xxx expected no of genotypes*

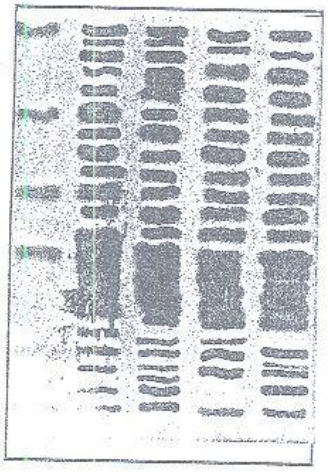
Table (3) :Correlation between genes frequency and semen characteristics of Arabian stallions

Semen characteristics	Al^F	Al^J	Tf^D	Tf^O	$Fo2^A$	$Fo2^B$	Es^G	Es^H	Ap^F	Ap^S	Gc^F	Gc^S
Ejaculate volume	-0.27*	0.27*	-0.16	0.16	-0.19	0.19	0.01	-0.01*	0.29	-0.29*	-0.27*	0.27*
Gell free volume	-0.26	0.26	0.01	-0.01	-0.01	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***	-0.82***	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	-0.02	0.62***	-0.62***
Sperm cell concentration	-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	-0.03
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 abnormalities	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 abnormalities	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	-0.08	0.23	-0.23

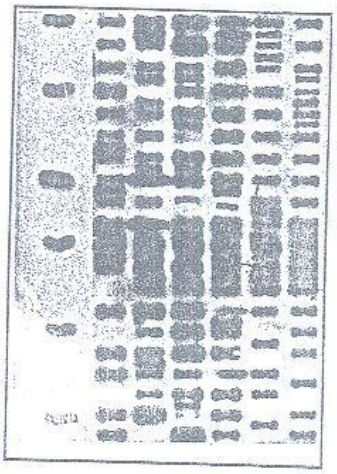
*p<0.05 significant correlation ** p<0.01 highly significant correlation *** p<0.001 very highly significant correlation



Fertile



Subfertile



infertile

Fig (1) Electrophoresis of plasma protein of Arabian stallions.