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IMMUNOGENETIC STUDIES IN RELATION TO FERTILE AND INFERTILE ARABIAN MARES

(With 6 Tables and 1 Plate)

By

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دراسة العلاقة بين دلالات المناعة الوراثية و التكاثر في الافراس العربية

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يهدف هذا البحث إلى الكشف عن العلاقة بين بعض دلالات المناعة الوراثية وبعض مؤشرات التكاثر وكذلك أيضا دراسة بعض الجينات الوراثية المتحكمة في النشاط الفسيولوجي و الخصوبة والكفاءة التناسلية في الأفراس العربية .أجريت الدراسة على عدد ٨٥ فرس عربية قسمت إلى ٩ مجموعات حسب الحالة التناسلية وقد تم تجميع عينات الدم و قياس مستوى هرمون البروجستيرون في المجموعات المختلفة كما تم استخدام التفريد الكهربي لتحديد المكونات الوراثية لعدد ٩ أنظمة من بروتينات البلازما و تم حساب معدل تردد الجين في هذه الانظمه في كل المجموعات محل الدراسه كما تم قياس معامل الخصوبه في هذه الحيوانات مرتبطا بالمكونات الوراثيه من ناحية و الحالة التناسلية من ناحية اخرى و قد اظهرت النتائج ان متوسط طول فترة العشار في الافراس العربية ٣٢٩,٣٤±٠,٩٨ يوم وكان معامل الخصوبه في الافراس السليمه تناسليا يتراوح من ٧١,٨٢ الى ٨١,٦٧ بينما في الافراس التي تعاني من مشاكل تناسليه (٥٩-٧٠) كما اوضحت الدراسه ان متوسط مستوى هرمون البروجستيرون في الافراس العشار ٠,٩٢ ± ٥,١١ نانوجرام/مل . وبالنسبه لتحاليل المناعه الوراثيه فقد اوضحت الدراسه ان الافراس غير العشار قد تميزت بسيادة الجينات التاليه Al^F, Es^H اماالافراس العشار فقد سادت فيها الجينات Pr^N, Pr^S, $F\alpha2^B$ أما الافراس التي كانت تعاني من مشاكل تناسليه فقد سادت فيها الجينات التاليه Al^F, Pr^N, $F\alpha2^A$. وأوضحت النتائج ان هناك ارتباط معنوي بين بعض الجينات الوراثيه و بين بعض الجوانب التناسليه فقد وجد ارتباط معنوي بين الجين Tr^O وطول مدة الحمل و الجين Es^G وطول مدة الحمل عندما يكون الجنين ذكر . كذلك وجدت علاقة ارتباطية معنويه ايجابيه بين الجين Pal^S ومستوى هرمون البروجستيرون. وقد خلص الباحثون إلى أن الخصوبة في

الافراس العربية تتحكم فيها مجموعة من الجينات يمكن الكشف عنها و استخدامها في التنبؤ المبكر بقيم الحيوان الانتاجيه وكفائته التناسليه.

SUMMARY

The present study aimed to investigate the possible correlations between genetic markers and reproductive patterns in Arabian mares, as well as to study the genetic control of some physiological activities associated with fertility and reproductive efficiency. The present study was carried out on 85 purebred Arabian mares kept at Al-Zahra Stud, Ain Shams, Cairo.Egypt. Mares were divided into 9 groups according to reproductive status, reproductive aspects, hormonal assay, as well as immunogenetic analysis were recorded. Results of current study revealed that non pregnant mares characterized by high frequency of Al^J genes during estrus and Es^H gene during diestrus while, pregnant mares revealed high frequency of Pr^N ,Pr^S and $F\alpha_2{}^B$ genes during early ,mid and late stages of pregnancy, respectively. Infertil mares revealed predominance of Al^F and $F\alpha_2{}^A$ genes in repeat breeder. Pr^N gene was more frequent in both cases of ovarian inactivity and persistent corpora lutea .There were highly significant correlations between the following genes GcF with $F\alpha_2{}^B$ and Ptf^R ,Gc^S with Ptf^N and Tf^O with Ptf^N .Results of correlations between genetic markers and reproductive parameters showed that Tf^O gene was highly significant correlated with gestation length and Pal^S with progesterone level .The correlation coefficients among reproductive parameters revealed highly significant correlation of number of females parity with age while fertility index was negatively correlated with age interval from foaling to conception.

Key words:*Immunogenetic studis, fertility, mares.*

INTRODUCTION

The Arabian horse are distributed nowadays in most countries of the world, because of its superior caliber and distinguished characteristics.

The genetic characteristics of the horse are classified into quantitative characters influenced by multiple minor genes and qualitative characters controlled by a single major gene.(Yoshizane and Tsutomu,1995).Genetic variation is the basis for differences in

performance and appearance between breeds and lines of horses, and improving of particular characteristics is possible by selective breeding from the superior animals. However, this type of selection is relatively crude and inefficient, and could be improved if more were known about the genes controlling the desired characteristics (Yoshizane and Tsutomu, 1995).

Introduction of new genetic markers in selection and breeding of Arabian horse is the main target, while principally within the last decade individual genes with large effects on reproduction have been identified and studied. These could not only contribute to increased understanding of the control of reproduction, but also, in some cases, have good potential for direct utilization to increase reproductive rates (Horni and Trakova, 1998; Vega *et al.*, 1998 and Budzynski *et al.*, 2000).

One of the most important goal of reproduction in equine is to detect the origin of provenance of species parentage especially pure breed ones, and the most effective polymorphic loci, which can reach this goal, are Pr and Tf loci (Braend and Storset, 1978) .The knowledge on underlying genetic basis for quantitative variation is still limited. However, relationship between quantitative traits and genetic markers can be used (Cizova *et al.*, 1998 and Albrecht *et al.*, 2001).Previous studies revealed significant correlations between reproductive parameters and genetic markers in mares (Mashurov ,1980;Lear *et al.*,1998 ;Bowling *et al.*,2000 and Albrecht *et al.*, 2001).

The present work aimed to investigate the possible correlations between genetic markers and reproductive parameters in Arabian mares under Egyptian condition, as well as to study the genetic control of some physiological activities associated with fertility and reproductive efficiency.

MATERIALS and METHODS

Animals:

The present study was carried out on 85 pure Arabian mares kept at Al-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 parasite, and a daily training to improve general health condition were practiced.

Based on the reproductive status, mares were classified into 9 groups as follows

Group 1: Fourteen fertile non-pregnant Arabian mares during estrus.
Group 2: Fifteen fertile non-pregnant control mares during diestrus.
Group 3: Thirteen early pregnant mares.
Group 4: Twelve mid pregnant mares.
Group 5: Twenty late pregnant mares.
Group 6: Four infertile repeat breeder mares during diestrus.
Group 7: Seven infertile repeat breeder mares during estrus.
Group 8: Seven infertile anoestrus Arabian mares suspected to have inactive ovaries.
Group 9: Three infertile anoestrus Arabian mares suspected to have persistent CL.

Reproductive aspects:

Breeding records including: history of breeding, number of parity, interval to foal heat, interval to conception, length of gestation period when the fetus was female, length of gestation period when the fetus was male and sex of offspring were analyzed External examination of the mares were done every day for the general body condition and the changes in the external organs. Estrus was detected by using proven teaser stallion for the non-pregnant mares.

Rectal examination of the mares was done to display the physiological functions and pathological conditions of the ovaries and tubular genitalia during the different stages of the reproductive cycle (Ataman *et al.*, 1999)

Fertility index was recorded (Ukalovic *et al.*, 1994) as follow:

$$FI = (n-1)\ 365x100/\ D$$

Where: FI = fertility index n = no. of offspring
D = Interval between 1st and last parturition (days)

Blood sampling:

Blood samples were collected from the jugular vein into clean dry sterile and heparinized vacutainer tubes during estrus and diestrus of fertile and infertile Arabian mares. Samples were also collected from the repeat breeder mares during estrus and diestrus, and from the anestrous mares that had inactive ovaries as well as from those proved to have persistent corpora lutea. All collected blood samples were centrifugated for 5 min. at 3000 r.p.m. Clear plasma were separated and stored in dry sterile labelled stoppered Eppendorff vials at – 20 °C.till being used for the hormonal assay and the immunogenetic analysis.

Hormonal assay:

Quantitative measurements of progesterone level in plasma were carried out using radio immunoassay (Metzger, 1992).

Immunogenetic analyses:

Electrophoretic pattern of plasma protein was performed by polyacrylamid gel electrophoresis (PAGE) (Carlstrom and Johnson, 1983).

Genetic markers:

Blood proteins loci included, Albumin (Al), Postalbumin (Pal), Pre-albumin (Pr), Transferrin (Tf), Post transferrin (Ptf), Serumcarboxylestrase (Es), α globulin (Fα2), Alkalinephosphatase (Ap), vit. D binding protein (Gc), were used as genetic markers in the present study. Distribution of genotypes and fractionation of blood protein were done according to Merkoreva (1977) and Mashurov (1980).

Determination of gene frequencies:

Determination of gene frequencies was estimated according to Hardi-Veinberg Law, Merkoreva (1977).

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

Where:

p^2 =homozygotic genotype AA

q^2 = homozygotic genotype BB

Statistical analysis of the data:

The obtained results were statistically analyzed according to Spiegel (1987 and 1988).

RESULTS

The age, parity, interval to foaling heat and foaling conception interval of Arabian mares were presented in Table (1) while, the gestation length in relation to sex of foal as well as the fertility index and plasma progesterone levels were recorded in Table (2).

The electrophoretic patterns of plasma proteins of Arabian mares during the various reproductive phases are shown in Plate (1).

Table (3) reveals the genes frequency of plasma protein loci in Arabian mares. It was found that non-pregnant mares revealed high frequency of Al^J gene (0.643) during estrus (group 1) and Es^H gene (0.7) during diestrus (group 2). On the other hand, in pregnant mares, Pr^N (0.73), Pr^S (0.73) and $F\alpha2^B$ (0.632) genes predominated during early, mid and late stages of pregnancy (groups 3, 4 and 5) respectively. In

infertile repeat breader mares (groups 6 and 7), Al^F (0.83) and $F\alpha2^A$ (0.74) genes predominated during diestrus and estrus, respectively. Infertile anoestrus mares (groups 8 and 9), Pr^N gene predominated in both cases of inactive ovaries (0.71) and persist corpura lutea (0.67).

Correlation coefficient, among various studied genes (Table 4) as well as among genes and fertility status (Table 5) were recorded. The most correlated genes were Gc^F with $F\alpha2^B$ and Ptf^R , Gc^S with Ptf^N and $F\alpha2^A$,Tf^O with Ptf^N and Tf^D with Ptf^R (Table 4). Highly significant correlation was found between Tf^O and gestation length as well as late gestation period in mares carrying female fetus. Also Es^G with late gestation period in mares carrying male fetus and Pal^S and progesterone level (Table 5). Moreover, correlation coefficient among reproductive parameters was recorded in (Table 6). Number of females parity increased with age, fertility index negatively correlated with age and with interval from foaling to conception.

DISSCUSION

The main goal of the present study was to characterize the immunogenetic constituents of Arabian mares with emphasis on relationships with some reproductive parameters.

The average length of gestation periods in Arabian mares in present study was 329.42 days, and it was significantly influenced by month of foaling, and not by sex of the fetus. This result comes in agreement with that recorded by El-Wishy *et al.* (1990).

The range of fertility index was 71 - 82 for the fertile Arabian mares where as in the infertile mares it was below this range (59 – 70) this finding is logic since such index depends upon the number of off springs as well as the interval between 1st and last foaling.

The present work indicated a significant increase in the plasma progesterone level in fertile mares during luteal phase than during follicular phase .A finding which came in consistent with that reported by Agag *et al.* (1996) for fertile mares and lower than that recorded by Stabenfeldt *et al.* (1971). Also, this finding came similar to that reported during the follicular phase by Abu Nawwara (2000).

The genetic relationship between blood protein loci and both productive and reproductive traits is based on protein coding loci (Barker *et al.*, 1997). In the present investigation all blood protein loci were found to be polymorphic, as the frequency of the most common allele was less than 0.95 (Kantanon *et al.*, 1999).

The present investigation used 9 polymorphic loci as a genetic markers to evaluate the reproductive efficiency of Arabian mares in Egypt .The most frequently genes were Al^{J} , Gc^{F}, Es^{G} and Tf^{O} .Genes frequency of Tf and Al was similar to results obtained by Han *et al (1998b)* for Cheju native Korean horse. Concerning Es locus the present study indicated 3 genotypes controlled by 2 autosomal alleles, while Cozzi *et al.* (1998) has recognized 4 variants of Es (F-G-I-S) in Sarcidano horses with high frequency of Es^{F} alleles (0.275), however the frequency of Es^{G} in this breed was similar to that obtained in the present study.

The current study indicated that, non pregnant mares during estrus were characterized by high frequency of Al^{J} , Ptf^{R} , Es^{G} and Gc^{F} genes, while mares during diestrus high frequency of Pr^{S} , Al^{J} , Pal^{S} , $F\alpha2^{A}$, Es^{H} , Ap^{F} and Gc^{F} genes was found, this variations indicated that each reproductive phase is controlled by a specific genes activity. In most species, the pre ovulatory surge of gonadotrpins begins approximately 24 hours before ovulation and is usually of short duration. The mares are an exception in that large amount of LH are released during an 8 to 9 days period with ovulation occurring on the third day, the high frequencies of Ptf^{R}, Es^{G} and GC^{F} in estrus of mares leads to probability of polygenic effect of these genes in ovulation in mares (Alexander and Irvine, 1986).

Concerning changes in progesterone level during pregnancy the present level during early stage was in agreement with the result of Irvine *et al.* (1999) in Thoroughbred mares and Abu Nawwara (2000) in Arabian mares. From immunogenetic point of view, the current study showed that each stage of pregnancy was characterized by high frequency of specific genes. These changes may be related to peculiarities of equine gestation in the form of formation of secondary CL between days 40 and 60 of gestation, the placenta synthesis of progesterone as early as day 50, and production of a gonadotropin called pregnant mare serum gonadotropin (PMSG).

Concerning of repeat breeder mares in the present study, a significant decrease in plasma progesterone level was found during the follicular phase similar finding was reported by Abu Nawwara (2000). Also, there were significant increase in the plasma progesterone level during luteal phase, a finding which indicated subnormal production of PGF2α due to chronic endometritis. From genetic view repeat breeder Arabian mares were characterized by high frequency of Pr^{N} gene. Similar result was detected by Pemberton *et al.* (1994) especially for the

association between N haplotype of α-II proteinase and endometritis in Thoroughbred mares. On the other hand, Weitkamp *et al.* (1980) studied the correlation between transferrin and esterase with the endometritis in mares, they were concluded that the presence of homozygotic (51%) and heterozygotic (34%) transferrin alleles was associated with pathogenic changes during the breeding season in fertile mares.

In infertile mares, the lowest and maximum plasma progesterone level, in the present study was observed for the anoestrous mares suspected to have inactive ovaries and persistent corpora lutea respectively. Similar results were reported by Townson *et al.* (1989) for anoestrus mares suspected to have persistent corpora lutea. Moreover; the infertile anoestrus mares with inactive ovaries were characterized by high frequency of Pr^N, $F\alpha2^B$ and Es^G genes. Where the mares had persistent CL were characterized by high frequency of Pr^N, Al^J, Tf^D, Ptf^R and Es^H genes. Concerning the last group of mares there was one highly frequent allele (PrN) these finding might explain the correlation between genetic markers and hormone receptors as Lori, E. Anderson *et al.* (2001) reported that the prostaglandin receptors FPr is genetically controlled. So we suggest that the FPr gene may be affected by Pr^N gene. Treatment with PGF2 decreased FPr mRNA expression in luteal cells in most species (Lori, E. Anderson *et al.*, 2001), therefore Pr^N may be also correlated to the PGF2 .

Concerning vitamin D binding protein (Gc), the present study indicated two autosomal alleles F and S that predominated during mid pregnancy (Gc^F), however it was reported that the frequency of this gene reveals species variations (Gronet *et al.*; 2000 and Sasimowski *et al.*; 2000).

The correlation among studied genes revealed a highly significant association between Gc^F with $F\alpha2^B$ and Ptf^R, Gc^S with Ptf^N and $F\alpha2^A$, Tf^O with Ptf^N and the condition may be due to closely connection of these genes on the same chromosome and the results were in line with the finding of Garalak *et al.* (2000). On the other hand, the reported correlations among some genes marker and some reproductive parameters such Tf^O and length of gestation period in mares carrying female feti, Es^G with mares carrying male feti and Pal^S with progesterone level. Such correlations indicated the possibility of genetic control of reproduction in mares and further investigations on their actual roles is still needed.

It could be concluded that the fertility index by Oelikokos equation has very highly significant positive correlation to the interval to

conception ($p<0.001$), TF^C ($p<0.05$) and TF^D ($p<0.05$) in Arabian mares. Progesterone levels of Arabian mares correlated positively $p<0.001$ to Pal^S and negatively to Pal^D. The immunogenetic constituents of Arabian mares reveled that all studied loci were polymorphic characterized by high frequencies for some genes, which vary according to the reproductive state.

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Table 1: The age, number of parity, interval to foal heat, interval from foaling to conception in different groups of Arabian mares.

(Mean ± S.E.)

Groups	N	Age (year)	N	Number of parity	N	Number of male parity	N	Number of female parity	N	Interval to foal heat(days)	N	Foaling to conception
1	14	13.89±1.15bc	14	7.71±0.72ab	14	4.20±0.49abc	14	4.21±0.55a	14	13.71±4.27ab	0	0c
2	5	14.40±0.19b	5	8.20±0.66a	5	4.00±0.80abc	5	4.40±0.98a	5	7.20±0.80b	0	0c
3	13	8.87±1.40d	13	5.31±0.89b	13	3.07±0.33cd	13	3.00±0.73a	13	13.23±6.41ab	13	71.77±6.41c
4	12	11.64±1.81cd	12	7.92±1.59ab	12	4.54±0.93abc	12	3.75±0.84a	12	23.42±12.47ab	12	54.92±12.49d
5	20	10.71±1.18cd	20	5.90±0.71b	20	2.80±0.27d	20	3.40±0.68a	20	14.15±5.58ab	20	47.80±6.89d
6	4	16.15±3.07abc	4	7.00±2.16ab	4	4.60±1.34abc	4	2.25±0.85a	4	6.50±0.50b	4	227±0.50a
7	7	12.42±2.83bc	7	5.71±1.86ab	7	3.13±0.97abcd	7	3.14±1.14a	6	15.83±8.08ab	6	125.50±8.09b
8	7	19.8±2.50a	7	8.14±1.39ab	7	4.63±0.64ab	7	3.86±1.10a	6	64.00±25.98a	6	0c
9	3	15.23±4.18abcd	3	6.67±2.03ab	3	3.00±1.00bcd	3	3.67±1.33a	3	87.00±49.83ab	3	0c

Means with different superscrips in each category are significantly differ at least at (p<0.05)

Table 2 : Length of gestation period in mares carried male or female feti , the fertility index and plasma progesterone level in different groups of Arabian mares. (Mean ± S.E.)

Groups	Length of gestation period (days)						N	Fertility index (%)	N	Progesterone Level (ng/ml)
	N	Female Feti	N	Male feti	N	Overall mean length				
1	8	321.63±3.47	6	327.17±1.89	14	324.00±2.21	14	72.51 ± 4.20BC	12	0.47± 0.12B
2	3	334.33±7.80	2	314.50±10.50	5	326.40±7.27	5	71.022 ± 7.4ABCD	5	6.69 ± 0.62A
3	5	338.80±1.32	8	333.00±2.62	13	334.46±1.72	13	79.01 ± 4.04AB	13	6.11 ± 0.94A
4	8	333.50±3.92	4	333.50±5.81	12	333.50±3.10	12	81.67 ± 3.08A	12	6.84 ± 0.92A
5	12	332.58±2.09	8	330.13±2.29	20	331.60±1.54	20	81.64 ± 3.64A	20	6.81 ± 0.88A
6	1	320	3	325.33±7.54	4	324.00±5.49	4	53.24 ± 6.06CD	4	4.83 ± 0.79A
7	3	328.67±2.91	3	325.67±2.96	6	327.17±1.97	6	70.74 ± 3.7BCD	6	0.66 ± 0.0.02B
8	2	324.50±1.50	5	329.40±2.77	7	328.00±2.14	7	59.83 ± 4.23D	6	0.35 ± 0.073B
9	1	316	2	326.00±1.00	3	322.00±3.06	3	60.06 ± 9.67CD	3	10.22 ± 2.5A

Means with different superscripts in each category are significantly at least at (p<0.05)

Table 3 : Genes frequency in different groups of Arabian mares

Protein loci	Genes*	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7		Group 8		Group 9	
		Gene frequency	X2	Gene frequency	X2	Gene frequency	X2	Gene frequency	X2	Gene frequency	X2	Gene frequency	X2	Gene frequency	X2	Gene frequency	X2	Gene frequency	X2
Pc	Pc^A	0.464	0.0003	0.4	0.14	0.73	3.64	0.27	3.65	0.575	0.24	0.375	1.36	0.71	0.69	0.71	1.39	0.67	1.34
	Pc^B	0.536		0.6		0.27		0.73		0.425		0.625		0.29		0.29		0.33	
Al	Al^A	0.357	2.546	0.4	0.14	0.42	0.61	0.55	0.052	0.5	0	0.83	1.02	0.5	0.145	0.43	0.21	0.33	1.34
	Al^B	0.643		0.6		0.58		0.45		0.5		0.17		0.5		0.57		0.67	
Pgd	Pgd^{11}	0.571	0.22	0.4	0.14	0.58	0.14	0.5	0	0.5	0.833	0.375	1.7	0.71	0.69	0.57	0.21	0.5	0.375
	Pgd^{8}	0.429		0.6		0.42		0.5		0.5		0.625		0.29		0.43		0.5	
Tf	Tf^{A}	0.536	6.684	0.5	2.81	0.39	10.33	0.33	0.204	0.5	0.033	0.625	1.36	0.43	3.7	0.5	3.42	0.67	1.66
	Tf^{D}	0.464		0.5		0.61		0.67		0.5		0.375		0.57		0.5		0.33	
Pgm	Pgm^{6}	0.607	0.916	0.5	2.81	0.42	4.91	0.17	1.45	0.6	3.168	0.625	1.36	0.57	0.21	0.57	1.45	0.67	1.66
	Pgm^{1}	0.393		0.5		0.58		0.83		0.4		0.375		0.43		0.43		0.33	
Ea2	$Ea2^{A}$	0.464	1.205	0.6	0.14	0.5	0.076	0.71	1.9	0.568	2.86	0.5	2	0.79	2.3	0.36	2.01	0.5	0.375
	$Ea2^{B}$	0.536		0.4		0.5		0.29		0.432		0.5		0.21		0.64		0.5	
Es	Es^{A}	0.607	5.56	0.3	1.815	0.54	2.81	0.64	2.83	0.6	0.56	0.375	1.7	0.29	1.39	0.64	0.031	0.33	1.34
	Es^{B}	0.393		0.7		0.46		0.36		0.4		0.625		0.71		0.36	7	0.67	
Ap	Ap^{A}	0.5	0.293	0.6	0.14	0.54	0.055	0.5	0	0.48	0.0018	0.375	1.36	0.36	0.0317	0.43	1.45	0.5	0.375
	Ap^{B}	0.5		0.4		0.46		0.5		0.52		0.625		0.64		0.57		0.5	
Gc	Gc^{1}	0.607	5.56	0.7	1.37	0.58	0.61	0.71	1.82	0.475	6.85	0.5	0	0.57	1.45	0.43	1.45	0.5	0.375
	Gc^{2}	0.393		0.3		0.42		0.29		0.525		0.5		0.43		0.57		0.5	

* International symbol of genes for each locus

Table 4 : Correlation coefficients among genes frequency in Arabian mares.

	Pr^N	Pr^S	Al^F	Al^I	Pal^D	Pal^S	Tr^D	Tr^G	Pif^R	Pif^N	$Fa2^A$	$Fa2^B$	Es^C	Es^H	Ap^F	Ap^S	Gc^D	Gc^S
Pr^N	1^^^	-1^^^	-0.36^^^	0.36^^^	0.58^^^	-0.58^^^	0.13	-0.13	0.48^^^	-0.48^^^	-0.34^^	0.34^^	-0.20	0.20	-0.21	0.21	0.64^^^	-0.64^^^
Pr^S	-1^^^	1^^^	0.36^^^	-0.36^^^	-0.58^^^	0.58^^^	-0.13	0.13	-0.48^^^	0.48^^^	0.34^^	-0.34^^^	0.20	-0.20	0.21	-0.21	-0.64^^^	0.64^^^
Al^F			1^^^	-1^^^	-0.41^^^	0.41^^^	-0.07	0.07	-0.20	0.20	0.20	-0.20	-0.11	0.11	-0.53^^^	0.53^^^	0.08	-0.08
Al^I			-1^^^	1^^^	0.41^^^	-0.41^^^	0.07	-0.07	0.20	-0.20	-0.20	0.20	0.11	-0.11	0.53^^^	-0.53^^^	-0.08	0.08
Pal^D					1^^^	-1^^^	-0.28^	0.28^	0.08	-0.08	0.26^	-0.26^	-0.05	0.05	-0.30^^	0.30^^	0.06	-0.06
Pal^S					-1^^^	1^^^	0.28^	-0.28^	-0.08	0.08	-0.26^	0.26^	0.05	-0.05	0.30^^	-0.30^^	-0.06	0.06
Tr^D							1^^^	-1^^^	0.87^^^	-0.87^^^	-0.55^^^	0.55^^^	-0.27^	0.27^	-0.24^	0.24^	0.55^^^	-0.55^^^
Tr^G							-1^^^	1^^^	-0.87^^^	0.87^^^	0.55^^^	-0.55^^^	0.27^	-0.27^	0.24^	-0.24^	-0.55^^^	0.55^^^
Pif^R									1^^^	-1^^^	-0.60^^^	0.60^^^	-0.27^	0.27^	-0.38^^^	0.38^^^	0.72^^^	-0.72^^^
Pif^N									-1^^^	1^^^	0.60^^^	-0.60^^^	0.27^	-0.27^	0.38^^^	-0.38^^^	-0.72^^^	0.72^^^
$Fa2^A$											1^^^	-1^^^	-0.45^^^	0.45^^^	-0.02	0.02	-0.74^^^	0.74^^^
$Fa2^B$											-1^^^	1^^^	0.45^^^	-0.45^^^	0.02	-0.02	0.74^^^	-0.74^^^
Es^C													1^^^	-1^^^	0.15	-0.15	0.08	-0.08
Es^H													-1^^^	1^^^	-0.15	0.15	-0.08	0.08
AP^F															1^^^	-1^^^	-0.56^^^	0.56^^^
AP^S															-1^^^	1^^^	0.56^^^	-0.56^^^
GC^F																	-1^^^	1^^^
GC^S																	1^^^	-1^^^

^ Significant correlation p<0.05
^^ Highly significant correlation p<0.01
^^^ Very highly significant correlation p<0.001

Table 5: Correlation coefficients among some reproductive parameters and genes frequency in Arabian mares.

Reproductive Parameter / Genes Frequency	AGE	Number of parity	Number of male parity	Number of female parity	Interval to foal heat (days)	Interval to Conception (days)	Length of Gestation (days)	Length of gestation with female fetus	Length of gestation with male fetus	Fertility index	Progesterone level (ng/ml)
Pr^R	-0.04	-0.20	-0.24*	-0.08	0.11	0.03	0.05	0.01	0.12	-0.11	-0.21
Pr^S	0.04	0.20	0.24*	0.08	-0.11	-0.03	-0.05	-0.01	-0.12	0.11	0.21
Al^F	-0.01	-0.02	0.11	-0.13	-0.09	-0.08	0.09	0.18	0.00	0.04	0.28*
Al^S	0.01	0.02	-0.11	0.13	0.09	0.08	-0.09	-0.18	0.00	-0.04	-0.28*
Pal^0	-0.05	-0.10	-0.16	-0.01	0.06	0.06	0.01	-0.13	0.17	-0.04	-0.55***
Pal^S	0.05	0.10	0.16	0.01	-0.06	-0.06	-0.01	0.13	-0.17	0.04	0.55***
Tr^S	0.26*	0.03	0.06	0.02	0.05	0.28*	-0.37**	-0.41***	-0.31**	-0.28*	-0.04
Tr^0	-0.26*	-0.03	-0.06	-0.02	-0.05	-0.28*	0.37**	0.41***	0.31**	0.28*	0.04
Ptt^R	0.16	-0.07	-0.08	-0.01	0.00	0.21	-0.29**	-0.30**	-0.26*	-0.21	-0.21
Ptt^S	-0.16	0.07	0.08	0.01	0.00	-0.21	0.29**	0.30**	0.26*	0.21	0.21
$Fa2^A$	-0.06	0.04	0.06	0.00	-0.06	-0.07	0.03	0.10	-0.07	0.03	-0.02
$Fa2^B$	0.06	-0.04	-0.06	0.00	0.06	0.07	-0.03	-0.10	0.07	-0.03	0.02
Es^G	-0.05	0.06	0.03	0.05	0.07	-0.07	0.18	0.03	0.36***	0.19	-0.08
Es^H	0.05	-0.06	-0.03	-0.05	-0.07	0.07	-0.18	-0.03	-0.36***	-0.19	0.08
Ap^F	-0.14	0.06	-0.02	0.09	-0.08	-0.10	0.09	0.16	0.02	0.14	0.22*
Ap^S	0.14	-0.06	0.02	-0.09	0.08	0.10	-0.09	-0.16	-0.02	-0.14	-0.22*
GC^F	-0.11	0.12	-0.10	0.08	-0.14	-0.09	0.05	0.10	0.04	0.14	0.06
GC^S	0.11	-0.12	0.10	-0.08	0.14	0.09	-0.05	-0.10	-0.04	-0.14	-0.06

* Significant correlation $p<0.05$
** Highly significant correlation $p<0.01$
*** Very highly significant correlation $p<0.001$

Table 6 : Correlation coefficients among some reproductive parameters in Arabian mares

	Age	No. o f parity	No. of male parity	No. o f female parity	Interval to foal heat	Interval to Conception	Length of Gestation	Length of gestation with female featus	Length of gestation with male featus	Fertility Index by Olikokos equation	Progesterone Concentration
Age	1.00***	0.88***	0.69***	0.74***	0.30**	0.45***	-0.16	-0.10	-0.22*	-0.39***	-0.09
No. of parity		1.00***	0.75***	0.85***	0.19	0.25*	-0.12	0.00	-0.26*	-0.04	0.01
No. of male parity			1.00***	0.29**	0.02	0.22*	-0.06	0.02	-0.13	-0.07	0.09
No. of female parity				1.00***	0.26*	0.20	-0.12	-0.01	-0.27*	-0.02	-0.07
Interval to foal heat					1.00***	0.30**	-0.07	-0.07	-0.08	-0.18	0.03
Interval to conception						1.00***	-0.11	-0.17	-0.03	-0.52***	-0.15
Length of gestation.							1.00***	1.00***	1.00***	0.05	0.22*
length of gestation with female featus								1.00***	0.00	0.16	0.26*
length of gestation with male featus									1.00***	-0.06	0.16
Fertility index by olikokos equation										1.00***	0.11
progesterone concentration											1.00***

* Significant correlation $p<0.05$
** Highly significant correlation $p<0.01$
*** Very highly significant correlation $p<0.001$

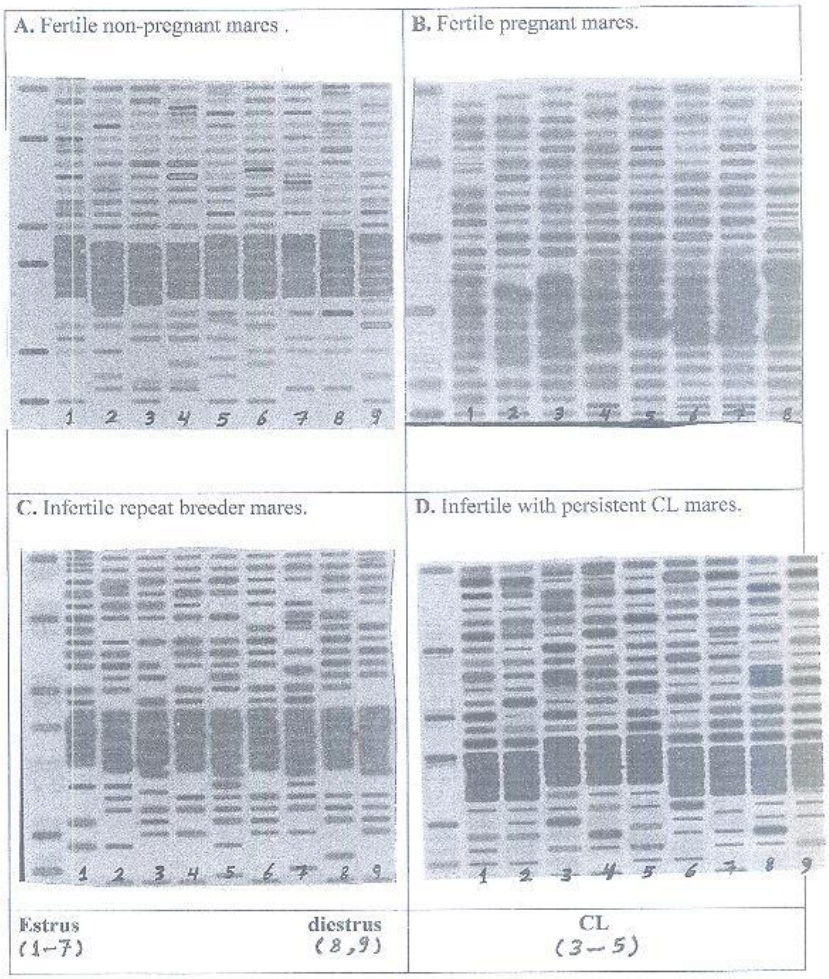

Plate (1) : Electrophoretic patterns of plasma proteins loci of fertile mares Fig. (A-B) and infertile mares Fig. (D-C).