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Relationship Between Immunogenetic markers and Some Reproductive Parameters in Purebred Arabian Mares

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Abstract: The present study aimed to evaluate some genetic markers of blood proteins and their relationship to some reproductive parameters in Purebred Arabian mare. Breeding records and data of Arabian mares have been collected and analysed regarding age of mares, number of parity, number of male parity, number of female parity, interval to foaling heat, interval to conception, length of gestation period with female/male fetus, length of gestation period with male fetus, overall length of gestation period in both fertile and low fertile mares. Gene frequencies and typing of both fertile and low fertile mare were done. Distribution of genotypes and fractionation of blood protein loci (Albumin (Al), Post albumin (Pal), Pre albumin (Pr), Transferrin (Tf), α-globulin ($F\alpha_2$),,Estrase (Es), vitamine D binding protein(Gc)) were carried out. Results elaborated that the average age (years), average interval to foal heat(days) and the average interval to conception(days) were 9.78±1.4, 15.6±6.41 and 54.24±7.8d for fertile mares while it was 13.65±2.63y, 38±18.54 and 155.5±8.5 for low fertile group. It was also found that the average length of gestation was significant ($P<0.05$) longer in fertile mares (331.6±5.22) than in low mares (322.07±4.14) while Fertility index was significantly ($P<0.05$) higher in fertile mares (75.67±3.96) than in low fertile mares (61.84±5.23). Results also showed high frequency of Al^{J}, of Ptr^{R}, Es^{G}, Gc^{F} genes in fertile Arabian mare while low fertile mares were characterized by high frequency of ofPr^{N}, $F\alpha_2{}^{B}$ and Es^{G}genes. Results also showed significant ($P<0.05$) positive correlation of Pr^{S} and negative correlation of Pr^{N} gene marker with number of male parity. Al^{F} was positively correlated with progesterone level, Tf^{D}gene marker was positively correlated with age and interval of conception, while it is negatively correlated with length of gestation in both male and female feti. Tf^{O} was positively correlated with the fertility index and predominated in mares having stable gestation in both and male and female feti. $Pal^{S}$was dominant in mares having high progesterone level while $Pal^{D}$was dominant in those having low progesterone level and low fertility index. Es^{G} was positively correlated with long gestation period with male feti while Es^{H}showed the reverse. This study concluded that the higher fertility of Purebred Arabian mares is tightly associated with high prevalence of Tf^{O}, $Pal^{S}$and Al^{F}gene markers.

Key words: Arabian Mare · Reproduction · Fertility index · Gene Markers

INTRODUCTION

Egypt is one of the most famous countries in the world in Arabian horse breeding. The mare is seasonally polyestrous with regular ovulatorycycles occurring in response to increasing day.

Fertility of mares is affected with genetic and environmental factors. Environmental factors include factors with measurable effects (Age, parity, foaling year, breeding season, etc) and those with un-measurable effects (Infectious diseases, parasitic infestations, etc) [1].

Gestation length in the mare is highly variable and it was reported between 332.84±0.81 and 336±0.5 days for Arabian mares [2,3]. In practice gestation lengths of 320-360 days are considered acceptable [4,5].Gestation length in the mare may be highly susceptible to both internal and external factors. Many external factors have

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been reported to affect gestation length in the mare. These may be considered as foetal, maternal or environmental in origin. Foetal factors include gender [5], weight [6]and breed [7]. Maternal factors include maternal age [8] parity [5], foaling to conception interval [9] and mating to ovulation interval [10]. Environmental factors include: month of conception or foaling [11], climate [12], nutrition of the mare [13] and year of foaling [14].

Immunogenetic analysis in horses has been investigated [15, 16]. Han *et al*., [17] has found in Chehu native Korean horses 14 variants of Transferrin (Tr) loci, 7 variants for Es loci, 3 variants for AI loci and 18 variants for phosphor-gluconate dehydrogenase (Phi) loci. Cozzi *et al.,* [18] have recognized 4 variants of Es (F-G-I-S) in Sarciano horses with high frequency of Es^Falleles (0.275).

The current study was designed to study the relationship of some blood protein genetic markers with parameters of fertility in Purebred Arabian mares.

MATERIALS AND METHODS

Animals: The present study was carried out on 85 Purebred Arabian mares kept at Al-Zahra stud. AinShams,Cairo-Egypt. Animals aged 3-21 years old. They were housed in closed stables with open yard for exercise and fed on balanced ration consisted of barley and Tibin with green fodders (Barssem or Darrawa). Mares have special care for diseases control, combating of external and internal parasite and a daily training to improve their general health condition.

Animal Grouping: Based on the fertility status, mares were divided into 2 groups, 1st group (fertile mares, n =64) and 2nd group (low fertile mares, n=21).

Detection of Estrus: Estrus was detected before blood sampling by using stallion to monitor the reflex of the mare to stallion. According to Ginther[19], estrus occurs when the mare was urinating and winking of vulva at any time during teasing as well as rising of tail up while being mounted.

Clinical Examination:

History of Breeding: Breeding records were investigated and data have been collected about age of mares, number of parity, number of male parity, number of female parity, interval to foaling heat, interval to conception, length of gestation period with female fetus, length of gestation period with male fetus and overall length of gestation period

Clinical Examination of Mares: Mares were examined for the general body condition and the daily changes in the external organs were recorded. Also, rectal examination of mares was done to display the ovarian and uterine status during the different stages of the reproductive cycle.

Fertility Index: Fertility index was calculated according to the formula of Ukalovic*et al*, [20].

$$F1 = (n-1)\ 365 \times 100 / D$$

Whereas:

n = Offspring's number

D = Interval between 1St and last parturitions (days)

Blood Sampling: Blood samples were collected from the jugular vein into clean dry sterile and heparinized vacationer tubes from 46 fertile and 21 low fertile mares, centrifuged for X1500g at 4° C for 15 minutes. Clean and clear plasma were aspirated carefully and kept in dry sterile Eppindorff vial sat -20°C till been used for the hormonal assay and the immunogenetic analysis.

Biochemical Analysis:

Hormaonal Assay: Progesterone level was estimated in plasma by ELISA kits from DIMA(Germany). The kit had a sensitivity of 2.0 pg/ml with inter- and intra- run precision coefficient of variations of 2.9 and 4.85, respectively[21]

Immunogenetic Analysis: Polyacrylamide gel electrophoresis (PAGE) was done according to Carlstrom and Johnson[22]

Genetic Markers(Blood Protein Loci): Albumin (Al), Post albumin (Pal), Pre albumin (Pr), Transferrin (Tf), α globulin ($F\alpha_2$), Estrase (Es), vitamin D binding protein (Gc).

Distribution of genotypes and fractionation of blood protein was done [23,24].

Statistical Analysis of the Data: The obtained results were statically analyzed according to Spiegel [25].

RESULTS

Results in Table 1 showed that the mean duration for pregnancy in the fertile marewas 331.60±5.22 days as compared with 322.07±4.14 days for the low fertile group.

Table 1: Some Reproductive parameters of Arabian mares.

Reproductive parameter	Fertile mares (N=64)	Infertile mares (N= 21)
Average age of dame (years)	9.78±1.4[a]	13.65±2.63[a]
Average number of male parity	3.45±0.68[a]	4.12±0.87[a]
Average number of female parity	3.75±0.68[a]	3.46±1.21[a]
Average interval to foaling heat(days)	15.60±6.41[a]	38±18.54[b]
Average interval to conception(days)	54.24±7.8[a]	155.50±8.5[b]
Average length of gestation(days)	331.60±5.22[a]	322.07±4.14[b]
Fertility index (%)	75.67±3.96[a]	61.84±5.23[b]
Plasma progesterone (ng/ml)	5.11±0.78[a]	2.80±0.25[b]

Means having different superscripts are significantly different at P<0.05

Table 2: Genotyping and gene expression of estrous and anestrous mares

Protein loci	Gene frequency in estrous mares N= 64	Gene frequengy in anestrous mares No=21
Pr	Pr^N 0.464	Pr^N 0.71
	Pr^S 0.536	Pr^S 0.29
AI	AI^F 0.357	AI^F 0.43
	AI^J 0.643	AI^J 0.57
Pal	Pal^D 0.571	Pal^D 0.57
	Pal^S 0.429	Pal^S 0.43
Tr	Tr^D 0.536	Tr^D 0.5
	Tr^G 0.464	Tr^G 0.5
Ptr	Ptr^R 0.607	Ptr^R 0.57
	Ptr^N 0.393	Ptr^N 0.43
$F_{ل2}$	$F_{ل2}^A$ 0.464	$F_{ل2}^A$ 0.36
	$F_{ل2}^B$ 0.536	$F_{ل2}^B$ 0.64
Es	Es^G 0.607	Es^G 0.64
	Es^H 0.393	Es^H 0.36
AP	AP^F 0.5	AP^F 0.43
	AP^S 0.5	AP^S 0.57
Gc	Gc^Fo.607	Gc^F 0.43
	Gc^S 0.393	Gc^S 0.57

Table 3: Correlation of some fertility parametrs with gene markers in purebred Arabian Mares.

	Reproductive parameter										
Gene Marker	Age	No of Parity	Number of Male Parity	Number of Female Parity	Interval to Foaling Heat	Interval to Conception	Length of Gestation	L.G.P.With Female Featus	L.G.P.With Male Featus	Fertilty index	Progesterone. Conc.
Pr^N	-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^D	-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***
Tf^D	0.26*	0.03	0.06	0.02	0.05	0.28*	-0.37***	-0.41***	-0.31***	-0.28*	-0.04
Tf^D	-0.26*	-0.03	-0.06	-0.02	-0.05	-0.28*	0.37***	0.41***	0.31***	0.28*	0.04
Fa_2^A	-0.06	0.04	0.06	0.00	-0.06	-0.07	0.03	0.10	-0.07	0.03	-0.02
Fa_2^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
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

* (P≤0.05) ** (P≤0.01) *** (P≤0.001)

The average age(years), average interval to foal heat(days) and the average interval to conception(days) were 9.78±1.4, 15.6±6.41 and 54.24±7.8 for fertile mares while it was 13.65±2.63, 38±18.54 and 155.5±8.5 for low fertile mares. The interval from foaling to conception was significantly (P<0.05) shorter in fertile mares (54.24±7.8) as compared to 155.50±8.5 in low fertile group.

It was also found that the average length of gestation (days) was significantly (P<0.05) longer in fertile estrous mares (331.6±5.22) than in low fertile mares (322.07±4.14). Fertility index and progesterone levels were significantly (P<0.05) higher in fertile mares (75.67±3.96 and 5.11±o.78) than in low fertile mares (61.84±5.23 and 2.80±0.25, respectively).

Results in Table 2 revealed high frequency of Al^{J}, of Ptr^{R}, Es^{G}, Gc^{F} genes in fertile Arabian mares in estrus while low fertile anestrous are characterized by high frequency of ofPr^{N}, $F\alpha_2{}^{B}$ and Es^{G}genes.

Results in Table 3 elucidated a significant (P<0.05) positive correlation of $Pr^{S}$and negative correlation of Pr^{N} gene marker with number of male parity. Al^{F} was positively correlated with progesterone level, Tf^{D}gene marker was positively correlated with age and interval of conception, while it is negatively correlated with length of gestation in both male and female feti.Tf^{O} was positively correlated with the fertility index and predominated in mares having longer gestation period in both and male and female feti. Also, it was recessive in mares having long intervals to conception. $Pal^{S}$was dominant in mares having high progesterone level while $Pal^{D}$was dominant in those having low progesterone level and low fertility. $Es^{G}$was positively correlated with long gestation period with male feti while Es^{H}showed positive correlation with long gestation period with female feti

DISCUSSION

The main purpose of the current investigation was to characterize the immunogenetic constituents of Purebred Arabian mares in relation to some reproductive parameters. The genetic relationship between blood protein loci and reproductive performance is based on protein coding loci [26]. The present study reported that the gestation length in Arabian mare is 331.60±5.22daysin fertilegroup as compared with 322.07±4.14 days for the low fertile mares. Results obtained in this study agree with the previous findings obtained by Howell and Rollins, [14], Rophia*et al*. [11] and Panchal*et al*. [5]. Unfortunately previous results are often complicated by other variables such as breed [7] and climate [11]. It is generally accepted that the male offspring of a range of species have longer gestations than female the reason is unclear [27]. It is postulated by some that the difference is due to different endocrine functions of male and female foetei interacting differently with the endocrine control of parturition [28].

In the current investigation, the average age for the fertile mare was 9.78±1.4 years as compared with 13.65±2.63 year for the low fertile group. Significant effect of age on gestation length was evident. This is in contrary with most of theother work but in agreement to others [6, 29] whose findings indicated an increase in gestation length with age. This was postulated to be due to a decrease in uterine/placental nutritional efficiency as a consequence of age and the multiparous state, slowing intrauterine growth and prolonging gestation [30].

In the current study, the interval to foal heat was significantly shorter (15.60±6.41)in estrous mares in comparison with anestrous mares (38±18.54). These results are in consistent with those findings got by El-Wishy*et al*. [31].

The effect of stallion age was similarly not significant. This is not surprising as there is no evidence to suggest that sperm viability, if indeed this is significantly affected by age, has any effect on gestation length of a viable conceptus. The interval from foaling to conception was significant shorter in estrous mares (54.24±7.8) compared to 155.50±8.5 in anestrous ones. These findings are agreement with El-Wishy*et al.* [31].

The present work indicated that the fertility index,accorrding to Oelikokos equation, was significant higher in fertile mares (75.67±3.96) in comparison with that in low fertile mares (61.84±5.23). The plasma progesterone concentrations were significant higher in fertile mares than those levels in low fertile group. These results are similar to those recorded by Agag *et al*., [32] and Abou Nawwara [33]who reported higher progesterone levels in fertile mares than anestrous ones.

Blood protein loci have been traditionally used in order to evaluate intra and interbreed genetic diversity in horse breeds [34,35]. There are twenty five blood plasma and red cell protein systems in horse. Some systems have been examined in Arabian horses while others, especially those routinely used in parentage testing have been studied in wide variety of breeds [34]. These standardized systems make it possible to compare results between the studies.

The current study elaborated that fertile Arabian mares showed high frequency of Al^{J}, of Ptr^{R}, $Es^{G}$and Gc^{F} genes while the low fertile mares were characterized by

high frequency of ofPr^N, $F\alpha_2{}^B$ and Es^Ggenes. The high frequency of Al^J, of Ptr^R, Es^GandGc^F genes in estrus indicates the probability of polygenic effect of those genes in ovulation of mares [36].Our present investigation revealed that the relationship between blood protein and steroid hormones is based on their great effect by plasma concentration of binding protein receptors. Ovarian steroid hormone stimulate RNA and protein synthesis indicating that those hormones act at the gene level through a receptor mediated mechanism [37].

Two autosomal alleles $Pr^N$and$Pr^S$of the prea;bumin (Pr) were identified in this study. In constant with this opinion, Pr was found in different species [37- 39]. In low fertile mares, there was one highly frequent allele named Pr^N. These finding might explains the genetic control of the hormone receptors [40].

It was also found that Tr locus has 2 alleles $Tr^D$and Tr^G. These alleles were also identified by Han *et al*.[17] in Cheju native horses. The results for genotyping of Gc in the present study are similar to those obtained by Weitkamp [41] who found 3 different phenotypes F, FS and S controlled by $Gc^F$and Gc^S alleles occured in Arabian horses.Similar results were previously recorded by Abu Atia [41].

In conclusion, It was found that the higher fertility of Arabian mares is tightly associated with high prevalence of Tf^O, $pal^S$and Al^Fgene markers. It was also found that $Tf^O$are positively related with high progesterone levels and high fertility index. On the other side Tf^D was dominant in older mares showing longer intervals to conception and shorter pregnancy course. Also Pal^D showed higher prevalence in low fertile mares having lower progesterone level.

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