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Observations on Some Blood Protein Genotypes and Sex Steroid Levels in Barki and Rahmany Ewes

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Abstract: The main objectives of this work was to investigate the immunogenetic differences between Rahmany and Barkiewes and to find the normal frequenciesof 3 blood protein genotypes(albumin- prealbumin-transferrin) at different reproductive stages and its inter-relationship with sex hormonal profiles. 17Barkiand 19 Rahmanyewes at different stages of reproductive cycle were used.Blood plasma was biochemically analyzed for blood protein polymorphism.Results revealed that blood protein genotypes varied between Barki and Rahmanyewes during the different stages of reproduction In non-pregnant ewes, the heterozygotes genotypes of the three studied loci were predominates in both species, with absolute predominates of Pr^F genetic allele in Barky ewes. While in Rahmany ewes the Alb^D and Pr^F showed high frequencies (75.0 and (62.3%, respectively). In pregnant ewes, the homozygotes genotype Al(DD), Tf (AA)were predominate with high frequencies of Al^D (72.7%) and Tf^A (77.3)%) in Barky ewes. In Rahmany ewes there was similar predominates of the homozygotes genotypes Al (DD0 and Tf (AA) with high frequencies of the gene markers Al^D (83.3) % and Tf^A (100)% respectively. Post-partum ewes distinguished by high frequencies of homozygotes genotypes Pr (FF) and Tf (AA) with gene frequencies Pr^F (100)% and Tf^A (75)% respectively for Barky ewes, and the same result for Rahmany ewes with regression frequencies (62.5)% of both alleles Pr^F and Tf^A. Concerning sex hormonal profiles, it was observed that estradiol - 17β concentration in blood plasma of Rahmanyewes averaged 3.91±0.92; 10.91±2.45 and 4.18±1.29 Pg/ml in nono-pregnant; pregnant andpost-partum respectively.While,it recorded a high basal level in Barki sheep (5.91 ± 0.99;14.57 ± 2.32 and 5.56 ± 1.05 Pg/ml, respectively).Moreover, progesterone levels were significantly high during pregnancy and averaged 3.88 ± 0.23 ng/ml, $P<0.05$ in Rahmany and 2.59 ± 0.93 ng/ml, $P<0.05$ in Barki sheep. It was concluded that, there are immunogenetic differences between Rahmany and Barki breeds represented in variations in the distribution and frequencies of blood protein genotypes which may be responsible for variations in the basal concentrations of steroid sex hormones and also fertility of sheep.Determination of blood protein frequencies can be used as a tool for predilection of reproductive efficiency of sheep.

Key words: Sheep · Electrophoresis · Estradiol · Progesterone · Blood proteins

INTRODUCTION

Sheep breeding is an important production field and common in many countries, which have arid climate conditions and wide pastures. With the advantage of characteristic traits of species, sheep breeding is highly profitable in low-quality pastures. Sheep is a livestock with good adaptability, easy handling, and low costs. Moreover, sheep can provide most of its nutritional needs from pasture Reproduction in sheep had received much attention in Egypt and most of developing countries either in the governmental or in the private sectors owing to these animals have high value as source of meat and wool.

Increasing the reproductive efficiency of native breeds of sheep such as Barki and Rahmany needs more investigations for identifications of the characteristic changes of the reproductive cycle including some hormonal aspects under the influence of different conditions.

There are many established factors that may affect the reproductive performance of ewes in the term of breed, age, season, health condition, nutrition and genetic constitutions. Using the immunogenetic methods

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specially blood protein polymorphism to evaluate and improve the reproductive efficiency in sheep were previously studied [1-7], It was reported that gene expression of blood protein could be considered as a tool for distinguishing between females during different reproduction phases. Also, they studied the estimation of genetic parameters for reproductive traits in different species of sheep. (Mehraban- Iranian fat tailed sheep-Iran-Black sheep) Moreover they used the Genome-Wide Association Study (GWAS) for more specific researches of reproductive traits of different sheep breeds[8-10].

Although the reproduction of native breeds of sheep is very reliable for meat and wool production, there is no enough attention for these breeds from genetics point of view. Therefor the objective of the current investigation was to determine the frequencies of 3 genotypes of polymorphic blood proteins with emphasis on their relation with sex hormonal profiles in different reproductive phases in some local Egyptian sheep breeds.

MATERIALS AND METHODS

Experimental Animals: Seventeen Barki and 19Rahmany ewes raised in a private farm, located in Sharkia governorate, Egypt were used in this study. The animals were apparently healthy, managed under routine condition and examined for detection of early pregnancy using Ultrasonography (Pia Medical Falcse`Saote, Netherlands). The animals were divided into three groups according to the reproductive status (non (pregnant, pregnant and post- partum).

Blood Samples: Blood samples were collected via vein puncture.Plasma was separated and kept at -20°C until analyzed for plasma protein and sex hormonal levels determination.

Analysis:

- Analysis of blood protein was carried out using starch gel electrophoresis according to the method of Lopez and Ramos [5].
- Gene frequencies and equilibrium: Gene frequencies was determined according to method of Hardi-Vainberg law[11].

Genetic equilibrium X^2 was determined by the law:

$$X^2 = \Sigma (f-F)2 / F$$

where f = number of observed genotypes, and F = number of expected genotypes.

- Determination of plasma estradiol-17β and progesterone levels were carried out using kits (Farmos Diagnos-tica Farmos Group, Ltd.)According to the methods of Abraham *et al.* [12] the radioactivity was measured under ã counter (Isotope Lab., NRC.)

Statistical Analysis: Data were analyzed statistically using analysis of variance ANOOVA according to Ropert and James [13].

RESULTS

Blood plasma were electrophoretically analyzed using starch gel electrophoresis, also, estradiol-17β and progesteronelevels in both breeds wee assayed the results showed that

Non-Pregnant Ewes: The heterozygotes genotypes of the three studied loci were predominates in both species with, absolute predominates of Pr^F genetic allele in Barky ewes. While in Rahmany ewes the Alb^D and Pr^F showed high frequencies (75.00 and (62.30%, respectively).

Estradiol-17β level averaged 5.91 ± 0.03 and 3.91 ± 0.92pg/ml in plasma of Barki and Rahmany ewes, respectively. While, progesterone level recorded 0.447 ± 0.03 and 0.104 ± 0.02ng/ml , respectively.

Pregnant Ewes: The homozygotes genotype Al(DD), Tf (AA) were predominate with high frequencies of Al^D (72.70%) and Tf^A (77.30%) in Barky ewes. In Rahmany ewes results revealed a similar predominates of the homozygotes genotypes Al(DD) and Tf (AA) with high frequencies of the gene markers Al^D (83.30 %) and Tf^A (100.00)%).

Estradiol-17β level averaged 14.56 ± 2.32 pg/ml in Barki and 10.91 ± 2.45pg/ml in Rahmany ewes, whereas, progesterone recorded a highly significant elevation during pregnancy in Rahmani (3.88 ± 0.23 ng/ml, $p<0.01$) and (2.59 ± 0.93 ng/ml, $p<0.05$) in Barki.

Post-Partum Ewes: Post-partum ewes were distinguished by high frequencies of homozygotes genotypes; Pr (FF) and Tf (AA) with gene frequencies; Pr^F (100.00%) and Tf^A (75.00%) for Barky ewes, respectively, and the same result for Rahmany ewes with regression frequencies (62.50)% of both alleles Pr^F and Tf^A.

Table 1: Genotyping and gene frequencies of blood protein loci of non pregnant, pregnant and post partum Barky ewes (N=17)

	Albumin					Pre-albumin					Transferrin				
Condition	DD	DF	FF	Frequency (%)	X^2	FF	FS	SS	Frequency (%)	X^2	AA	AB	BB	Frequency (%)	X^2
NonPregnant	1	1	--	Alb^D =75.0 Alb^F =25.0	0.16	---	2	---	Pr^F=50.0 Pr^S=50.0	2.0	---	2	---	Tf^A=50.0 Tf^B=50.0	2.0
Pregnant	7	2	2	Alb^D =72.7 Alb^F =27.3	3.35	5	6	--	Pr^F=72.7 Pr^S=27.3	1.49	8	1	2	Tf^A=77.3 Tf^B=22.3	5.66
.															
Post Partum	---	2	2	Alb^D =25.0 Alb^F =75.0	0.48	4	---	---	Pr^F=1.00 Pr^S=0.00	0.0	2	2	---	Tf^A=75.0 Tf^B=25.0	0.48

Table 2: Genotyping and gene frequencies of blood protein loci of non pregnant, pregnant and post partum Rahmany ewes (N=19)

	Albumin					Pre-albumin					Transferrin				
Condition	DD	DF	FF	Frequency (%)	X^2	FF	FS	SS	Frequency (%)	X^2	AA	AB	BB	Frequency (%)	X^2
NonPregnant	4	2	1	Alb^D =75.00 Alb^F =25.00	0.88	2	2	4	Pr^F=37.70 Pr^S=62.30	1.80	1	3	4	Tf^A=31.20 Tf^B=68.80	0.12
Pregnant	5	1	2	Alb^D =83.30 Alb^F =16.70	0.113	---	3	--	Pr^F=50.00 Pr^S=50.00	3.00	3	---	---	Tf^A=1.00 Tf^B=0.00	---
.															
Post Partum	1	2	1	Alb^D =50.00 Alb^F =50.00	0.00	2	1	1	Pr^F=62.50 Pr^S=37.50	0.90	2	1	1	Tf^A=62.50 Tf^B=37.50	0.90

Estradiol-17β and progesterone levels averaged 5.56 ± 1.05 pg/ml and 1.02 ± 0.43 ng/ml in Barki and 4.18 ± 1.29 pg/ml and 0.08 ± 0.03 ng/ml in Rahmany ewes, respectively.

DISCUSSION

The percentage of albumin content in blood ranged between 3.5-5.0 g/dl. Its low molecular weight (66241 M.W.) gives ability to pass faster than molecular weight contents of serum proteins during electrophoresis.The polymorphism of serum albumin gives about 20types of albumin which are controlled by 6 autosomal co dominant alleles (D, F, S, T, V and W) [6, 9, 14].

Transferrin is considered meta-protein of serum, and it plays essential role in binding and carrying Fe^{+++} in the body also it plays a significant role as antibacterrin in sheep averaged 77,000 KD [15]. In addition, transferrin is considered fractions of β l-globulin and its level in plasma ranged be-tween 200-300 Mg% or 10% of serum protein of sheep. Electrophoresis of transferrin either with starch gel or polyacrylamide gel give 13 different types of transferrin in sheep [3].

In the present study, variation in the distribution of blood protein genotypes and frequency of different alleles during the different reproductive stages between Barki and Rahmany ewes was found. These variations in the frequencies of different alleles between two different breeds and among the different reproductive stages coordinate with the finding of many investigators, who reported that there is substantial genetic variation in ovulation rate in multiple ovulating animal species between and within breeds due to apparently to segregation of major genes [2, 4, 12-16]. The high frequency of Tf^A in the present study was similar to those result previously reported [6] while results of polymorphic system of Albumin gives different results according species with different results of frequency of Alb^A and Alb^B [17], whereas other authors found no polymorphism of this system [18, 19].

Concerning steroid , these are major regulators of uterine and placental growth and function in mammalian species . Steroid hormones exert their direct effects through nuclear and 44 membrane receptors [20]. Uterine and placental expression of estrogen and progesterone progesterone level during pregnancy in Rahmany ewes, our findings were similar to result obtained by other author [21].

Steroid and thyroid hormones are lipophitic enough to diffuse across the cell membrane of all cells. Ovarian steroid hormones stimulate RNA and protein synthesis lead to concept that these hormones acting at the gene

level through a receptor-mediate mechanism [22]. The interaction between receptors and steroid hormones cause a change in the conformational structure of the receptors which called receptor activation and when the steroid binds to receptor the DNA binding region becomes exposed and the receptor affinity for the DNA increases the steroid – R complex binds to specific segment of DNA which determines which genes express themselves. So, the result of this work confirm this phenomena, though the correlation between genes which controlled three serum proteins and steroid hormones. Schrader *et al.* [23] suggested that the α-subunit of progesterone receptor binds to DNA and the β-subunit binds to chromatin. In the present study it was observed that protein fraction Alb-FF was associated with an increase in the level of estradiol-17β (10.02 ± 4.9 pg/ml), whereas, TfBB, was associated with decrease in the progesterone levels (0.08 ± 0.04pg/ml). This finding explains a relationship between some blood protein fractions (genotype) and activity of steroid hormones. The manipulation of single locus could therefore be highly relevant in circumstances such as those in which decreased levels of the product decrease negative feedback on the target system. The feed-back control of reproduction of ovarian hormones controlling the release of FSH would be likely to increase the ovulation of sheep [2425].

It was concluded that, there are immunogenetic differences between Rahmany and Barki Egyptian local breeds of ewes represented in variations in the distribution and frequencies of blood protein genotypes which may be responsible for variations in the basal concentrations of steroid sex hormones and also fertility of ewes. Determination of blood protein frequencies can be used as a tool for predilection of reproductive efficiency of sheep.

REFERENCES

1. Zaabal, M.M., 1997. Evaluation of some reproductive patterns of native breed of sheep using blood protein genotypes and sex hormonal levels. Zag. Vet. J., 25(1): 60-66.
2. Hanford, K.J., L. D.VanVleck and G.D. Snowder, 2003. Estimates of genetic parameters and genetic change for reproduction, weight, and wool characteristics of Targhee sheep. J. Anim. Sci., 81: 630-640.
3. Pramod, K.R., S.K. Sharma, R. Kumar and A. Rajan, 2013. Genetics of ovulation rate in farm animals. Veterinary World, 6(11): 833-838.
4. Fesus, L., J. Varkony and A. Ats, 1983. Biochemical polymorphism in goat with special reference to the Hungarian native breed Animals. Blood groups Biochem. Genet., 14: 1-6.
5. Lopez-Galves, G., M. Juarez and M. Ramos, 1995. Two dimensional electrophoresis and immunoblotting for the study of ovine whey protein polymorphism. J. Dairy Res., (62)2: 320-331.
6. Hrincã, G. and P.G. Vicovan, 2009. Comparative analysis of the hemoglobin system in the Romanian sheep breeds. Annals of RSCB., 2: 32-40.
7. Yavarifard, R., N. Ghavi Hossein-Zadeh and A.A. Shadparvar, 2015. Estimation of genetic parameters for reproductive traits in Mehraban sheep. Czech J. Anim. Sci., 60: 281-288.
8. Abdoli, R., S. Mirhoseini, N. Ghavi Hossein-Zadeh, P. Zamani, M. Ferdosi and C. Gondro, 2019. Genome-wide association study of four composite reproductive traits in Iranian fat-tailed sheep. Reprod. Fertil. Dev., 31: 1127-1133.
9. Salah Faraj, 2019. Genetic polymorphism of Some Biological Parameters in Arabi Sheep Blood and Relation with adaptation capability. A thesis for a degree of Master in Agriculture Sciences. Dep. of Animal Resources (Animal Breeding and Genetics). College of Agriculture, Univ. of Basrah.
10. Hasan Baneh, Javid Ahmadpanah and Yahya Mohammadi, 2020. Genetic analysis of Reproduction characteristics in Iranian-Black Sheep. Animal production. Acta Sci Anim. Sci., pp: 42.
11. Merkoreva, E.K., 1977. Genetic base of selec-tion in foam Moscow, Koloc, 121.
12. Abraham, G.E., 1981. The application of natural steroid radioimmunoassay to gynecologic endocrinology. In "Radioassay Systems in Clinical Endocrinology", Abraham, G.E. (ed), Marcel-Dekker, Basel, pp: 783-800.
13. Ropert, G.D. and H.T. James, 1960. Principles of statistics special references to biological sciences. M.C. Graw-Hill Book company, Inc. Publisher NY Toronto, 99.
14. Nesse, L.L. and H.J. Larsen, 1986. A practi-cal method for production of all-antibodies against goat lymphocytes antigens. XXth, International Conference on Animal blood groups and Bichemical polymorphism. 28/7-1/8/1986. Helsinki, 35.
15. Spooner, R.L., R.A. Oliver and N. Richarson, 1975. Isolation and partial characeriza-tion of sheep transferring. Comp. Biochem. Physiol., 52: 515-522.

16. Kamjoo, B., H. Baneh, V. Yousefi, A. Mandal and G. Rahimi, 2014.Genetic parameter estimates for growth traits in Iran-Black sheep. Journal of Applied Animal Research, 42(1): 79-88.
17. Ostrhoff, D.R. and I.C. Ward-cox, 1970. Serum polymorphism in three south African goat breeds - Proc. 12 th Eur. Conf. blood groups biochem. Polymorphism. Budapest, pp: 579-582.
18. Efremov, G. and M. Braend, 1964. Haemo globins, transferrins and albumins of sheep and goats. Pocecding of 9th European conf. on Anim. Blood groups. (Prague), pp: 313-320.
19. Fesus L.J. Varkoyi and A. Ats, 1983. Bio-chemical polymorphism in goat with special reference to the Hungarian native bread Animal. Blood groups Biochem. Genet., 14: 1-6.
20. Albrecht, E.D. and G.J. Pepe, 2015. Placental endocrine function and hormone action. In Plant TM, 315 Zeleznik A.J., eds. The Knobil and Neill's Physiology of Reproduction, edn 4, New York, 316 NY: Academic Press as an imprint of Elsevier; 2015: 1783-834.
21. Omar A. Salama, 1991. Studies in reproduce-tive physiology of sheep. Ph.D. thesis, Faculty of Agriculture, Cairo University, Egypt.
22. Clark, J.H. ,1977. Reproduction in Domestic Animals. H.H. Cole and Cupps. Eds, 143, Academic Press, New York.
23. Schrader, W.T., D.O. Toft and B.W. O'Malley, 1972. J. Biol. Chem. 247 (Reproduction in Domestic animals, Perry T. & Cupps), 4th Edition, 1991, Academic Press, pp: 105-110.
24. Scaramuzzi, R.J. and R.M. Hoskinson, 1984. Active immunization against steroid hor-mones for increasing fecundity. In Immu-mals, pp: 445-474 (Ed., D.B. Crighton. Butterworth's, London).
25. Webb, R., R.B. Land and N. Pathiraja, 1984. Passive immunization against steroid hormones in the female. In Immunological As-pects of Reproduction, Butterworths, London.