Study on Twinning in Some Local Breeds of KSA Sheep

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Abstract: Thirty two ewes of the two sheep breeds (Harri and Najdi) were used at Alkhurmah Governorate, Kingdom of Saudi Arabia (KSA). The animals were chosen according to their prolificacy (i.e. number of lamps born per parturition per female). Ewes of each breed (sixteen ewes) were divided into two groups representing the prolific and non-prolific females. Their blood samples were examined for protein polymorphism using the SDS-PAGE technique, and also for the DNA polymorphism using the RAPD-PCR technique. Analysis of variance and least squares means for the traits indicated that breed had significant effect on all studied traits except lamping interval and kilograms of lamps weaned per ewe. However, level of fecundity within each breed affected significantly only litter size and twinning rate as well as kilograms of lamps lamped per ewe. According to protein polymorphism data, each of the Harri and Najdi females had different specific protein markers at molecular weights ranging from 28 to 179 KDa for the Harri breed, and from 42 to 162 KDa for the Najdi one. The relationship between these traits and the genetic markers was also considered. The results showed also that the RAPD-PCR technique was able to separate with precision between Harri and Najdi individuals using some specific bands of different molecular weights. The associations between the blood protein banding patterns generated by the SDS-PAGE technique and the traits showed that the existed associations varied according to level of prolificacy within each breed and suggested that marker assisted selection could be carried out at early ages for the improvement of reproductive and productive traits in Harri and Najdi sheep.
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1. Introduction

Determination of the genetic diversity of indigenous sheep in respect to these important economic genes has not been sufficiently studied. Genetic characterization and determination of genetic differences between sheep breeds will help in the genetic improvement programs **(Crawford and Littlejohn, 1998; Almahdy *et al.*, 2000b, Kumar *et al.*, 2006; EL Hanafy and Salem, 2009)**. Large genetic variations in the twining rate and litter size have been observed among different breeds and within breeds of sheep. In some instances, variation in the litter size and ovulation rate can be genetically controlled by the action of a single gene with a major effect, named fecundity gene **(Davis, 2005)**. Three types of fecundity gene have been reported in sheep, namely Bone Morphogenetic Protein Receptor IB (BMPR-IB) also known as Booroola fecundity gene (FecB) **(Wilson *et al.*, 2001)**.

Litter size and lamb growth are important economic values in sheep breeding and reproduction. Recently, **Mulsant *et al.* (2001), Souza *et al.* (2001) and Wilson *et al.* (2001)** reported that bone morphogenetic protein receptor IB (BMPRIB) gene mutation was responsible for the high prolificacy associated with the FecB gene in Booroola Merino sheep. This mutation is located in the kinase highly conserved domain of the bone morphogenetic protein receptor IB, and is characterized by 'precocious' differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles **(Souza *et al.*, 2003)**. This mutation can be detected directly by forced PCR restriction fragment length polymorphism (RFLP) approach based on the reports described by **Souza *et al.* (2001) and Davis *et al.* (2002)**. PCR-RFLP is a rapid, simple and exact technique for single nucleotide polymorphism (SNP) genotyping. This approach has been used previously to genotype prolific sheep and goat by several research groups **(Souza *et al.*,2001; Davis *et al.*, 2002; Kumar *et al.*,2006; Guan *et al.*,2007; Polley *et al.*, 2009)**. Phylogenetic analysis revealed that the nucleotide sequences of alleles observed in the present study and that of a published sequence of sheep were having the same point of origin. The results were also compared with goats, large ruminants and humans. The allelic frequencies of allele A and B were 0.64 and 0.36, respectively in Corriedale sheep whereas the allelic frequencies of

all the three alleles in Kashmir Valley sheep were 0.60, 0.34 and 0.06 **(Shabir *et al.*, 2013)**.

Thus, This study aimed to identify the two local sheep breeds (Harri and Najdi) to study the homogeneity of these breeds (prolific and non prolific) at the biochemical genetic level (protein polymorphism) and molecular genetic level (DNA analysis) and to Find molecular markers, which can differentiate between breeds and help for future breeding programs.

2. Materials and Methods

2-1- Animals and management

Two sheep local breeds (Harri and Najdi) were used at Alkhurmah Governorate in Saudi Arabia kingdom (KSA). Sixteen animals of each breed were chosen according to the prolificacy trait (i.e. number of lamps born per parturition per female) and grouped in two sub-groups represented prolific (i.e. two lamps or more) and non-prolific (i.e. one lamp) ewes. All animals were managed under the same conditions and they were healthy and in a good body condition and were fed *ad libitum*, while animals were allowed to drink water two or three times per day according to the ambient temperature. Sheep were housed in semi-open sheds.

Then, two groups of measurements were collected for each ewe:

2-1-1- Productive measurements:

The following productive data were collected as follows:

Kg born / ewe lamped (KB): the number of kilograms of lamps born for each ewe lamped.

Kg weaned / ewe lamped (KW): the number of kilograms of lamps weaned for each ewe lamped.

2-1-3- Reproductive measurements:

The following reproductive data were collected as follows:

Ewe age at first lamping (AFL) in days.

lamping interval.

Litter size (LS): the number of lamps born / ewe lamped (single, twins, ….etc).

Twinning rate (TR).

2-1-3- Statistical analysis of the productive and reproductive measurements:

Data were analyzed using the General Linear Model procedure (GLM) of SAS (2004). Sources of variations were tested through analysis of variance procedure and mean differences were separated according to Tukey (1961). The statistical model used to describe data was as follows:

$Yijk = \mu + Bi + (GB)ij + eijk$

Where:

Yijk: the measurements of productive or reproductive traits on the lth animal of the ith breed of the jth group nested within the ith breed;

μ: the overall mean;

Bi: the fixed effect of the ith breed (i = 1,2; i.e. 1= Najdi, 2= Harri);

(GB)ij: the fixed effect of the jth group nested within the ith breed (j = 1,2; i.e. 1= prolific, 2= non-prolific);

eijk: the random errors assumed to be normally distributed with mean = 0 and variance = δ2 e.

2-2- Blood samples collection

Blood samples were collected from the animals by vacutainer glass tube contained disodium EDTA (EDTA-Na2) as anticoagulant reagent. Blood plasma was then obtained by centrifugation at 5000 rpm for 15 minutes at 4ºC, and the plasma protein (supernatant) and clots were transferred to clean plastic vials and stored at -20ºC until the time of laboratory analyses, however, the pellet was stored at -20°C until the time of DNA extraction.

2-3- Protein electrophoresis using SDS-PAGE technique

Samples were applied to 15% polyacrylamid gel. Gel preparation, electrophoresis condition, staining and destaining gels were done according to Laemmli (1970). Protein fractionation was performed exclusively on vertical slab (19.8 cm × 26.8 cm × 0.2 cm) gel using the electrophoresis apparatus manufactured by Aplex.

2-4- DNA electrophoresis using RAPD-PCR technique

DNA was isolated with the phenol-chloroform extraction method as described by Sambrook *et al.* (1989). The amplification conditions were carried according to Williams et al. (1990). Extracted DNA was then quantified using the UV spectrophotometer (Eppendorf BioPhotometer). Gel was stained with 0.2 μg/ml ethidium bromide and PCR products were photographed and scanned by gel documentation system (Gel Doc. BIORAD 2000) under UV transilluminator and analyzed with the Quantity One Software package supplied by the manufacturer.The random amplified polymorphic DNA-RAPD technique consists in the amplification, by polymerase chain reaction (PCR), of random segments of genomic DNA using a single short primer of arbitrary sequence, (Callejas and Ochando, 1998). The phenotypic correlation coefficients between each of productive and reproductive traits and protein banding patterns were calculated and tested for significance according to Snedecor and Cochran (1967).

3. Results and Discussion:-

Productive and reproductive traits:

Productive and reproductive performance of Harri and Najdi sheep are shown in table 1. Harri sheep tended to give h kilograms of lamps at lamping (3.63 vs. 4.14) and more kilograms of lamps at weaning (24.58 vs. 20.13) compared to Najdi sheep. This may be explained by the higher litter size (1.55 vs. 1.42) and twining rate (1.72 vs. 1.45) in the case of Harri sheep compared to Najdi sheep(Table 1). Moreover, age at first lamping and lamping interval are higher in Najdi sheep compared to Harri sheep. This means that Najdi sheep is late mature and has low mothering effect throw suckling period than Harri sheep. In the two sheep breeds, twining rate and litter size are greater in prolific females compared to the non-prolific ones. Consequently, prolific females tended to give more kilograms of lamps both at lamping and weaning compared to non-prolific ones.

Analysis of variance and least squares means for the traits considered in the present study indicated that breed had significant effect on all studied traits except lamping interval and kilograms of lamps weaned per ewe. However, level of fecundity within each breed affected significantly only litter size and twinning rate as well as kilograms of lamps lamped per ewe.

Table 1: Overall means and standard errors (±) for productive and reproductive traits of Harri and Najdi breeds.

Traits	Harri prolific females	Harri non-prolific females	Harri as a whole (Prolific + Non-prolific)	Najdi prolific females	Najdi non-prolific females	Najdi as a whole (Prolific + Non-prolific)
Age at first lamping (days)	513.26±47.53	497.18±34.95	504.06±22.47	842.0±44.76	727.0±53.89	784.62±34.89
Twinning rate	2.34±0.08	1.07±0.04	1.72±0.05	1.68±0.03	1.05±0.08	1.45±0.05
Litter size	2.11±0.13	1.00±0.01	1.55±00.13	1.92±±0.34	0.91±0.06	1.42±0.13
Lamping interval (days)	387.27±25.46	334.00±47.35	360.64±23.09	469.87±44.37	423.33±34.60	446.13±26.01
Kg. lamps at lamping/ewe	3.4±0.21	3.86±0.34	3.63±0.15	4.41±0.56	3.86±.0±43	4.14±0.89
Kg. lamps at weaning/ewe	23.47±1.83	25.64±1.37	24.58±1.41	19.64±2.29	20.62±0.87	20.13±1.37

Least squares means of the traits considered in the present study showed that Najdi sheep breed had late age at first lamping and lower kilograms of lamps lamped per ewe than Harri sheep. However, Harri females showed higher litter size and twinning rate compared to Najdi females. On the other hand, within each breed the prolific females tended to be smaller in size, early mature and give more lamps at birth and more kilograms of lamps weaned per ewe.

Protein polymorphisms:

The serum protein samples for Harri (H) and Najidi (N) sheep were separated using SDS-PAGE technique in two breeds. Each breed was divided into two groups. The first group (samples from 1 to 8-A) was classified as prolific animals while the second group (samples from 1 to 8-B) was classified as non-prolific animals based on the animal performance according to number of lamps born per parturition per female (two lamps or more for the first group and one kid for the second group).

Sixteen individuals from Harri population were sampled. Their protein electrophoretic banding patterns are shown previously in Figure (1A) for the prolific group (HP) and Figure (1B) for the non-prolific one (HN). A total of 11 bands for HP group and 8 bands for HN group were labeled according to their relative fronts based on the band molecular weight along the gel.

According to Figure (1A and 1B) the range of molecular weight (MW) of protein bands was from 37.1 to 243.4 KDa for HP group, and from 38.0 to 244.2 KDa for HN group.

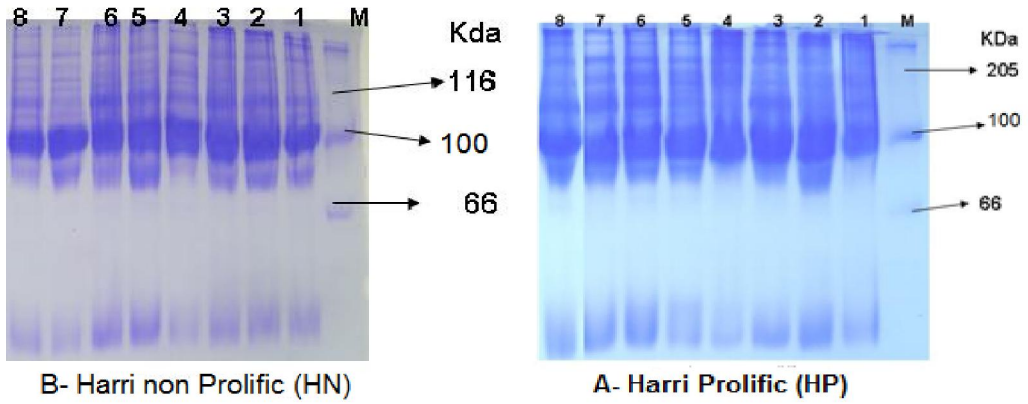

Figure (1): Protein electrophoretic banding patterns for Harri sheep.

Also, sixteen individuals from Najdi population were sampled. Their protein electrophoretic banding patterns are shown in Figure (2A) for the prolific group (NP) and Figure (2B) for the non-prolific one (NN). A total of 17 bands for NP group and 10 bands for NN group were labeled according to their relative fronts based on the band molecular weight along the gel.

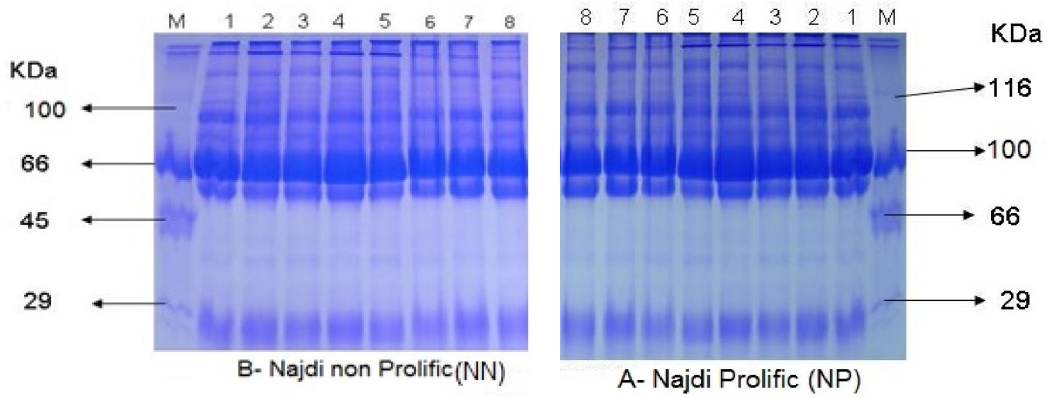

Figure (2): Protein electrophoretic banding patterns for Najdi sheep.

According to Figures (2A and 2B) the range of protein bands was from 41.6 to 162.5 KDa for NP group, while it was from 21.0 to 143.9 KDa for NN group.

From the previous figures the protein electrophoresis (SDS-PAGE) in the present study indicated that each sheep population had a unique banding pattern. Thus, we can conclude that SDS-PAGE is a sensitive method for studying the genetic structure of sheep populations. Many electrophoretic methods such as isoelectric focusing (Machado ***et al.,*** 2000), and SDS-PAGE (Laemmli, 1970) were used by different investigators to separate animal plasma and serum proteins for detecting biochemical genetic markers.

3) RAPD polymorphisms analysis

In the present work, genetic variation between and also within each one of the two sheep breeds (Harri and Najdi), based on RAPD-PCR analysis, have been examined to evaluate the homogeneity between and within each population and also to find genetic markers characterize each breed. Generally, many researchers were employed the Random amplified polymorphic DNA markers technique to characterized and estimate genetic distances between sheep breeds (Williams *et al.,*1990; Welsh and Mecldhmd, 1990; Nyamsamba *et al.,*2002; Ouafi *et al.,* 2002), in the study of genetic diversity within breeds (Li *et al.,*2002; El-Seoudy *et al.,*2005) and in determination of sex and gene mapping in farm animals (Vaiman *et al.,*1996).

The blood samples of Harri sheep breed were separated in two groups using RAPD-DNA technique. The first group (samples from 1 to 8) was classified as prolific animals while the second group (samples from 9 to 16) was classified as non-prolific animals according to number of lamps born per parturition per female.

RAPD fingerprints of individual samples for Harri sheep breed. M = DNA marker. Lanes 1-8 represent non-prolific females and lanes 9-16 represent prolific female

Sixteen individuals from Najdi population were sampled and analyzed. The first group (samples from 1 to 8) was classified as prolific animals while the second group (samples from 9 to 16) was classified as non-prolific animals according to number of lamps born per parturition per female. The results of their PCR profiles generated by the different primers are described in the following.

RAPD fingerprints of individual samples for Najdi sheep breed. M = DNA marker. Lanes 1-8 represent non-prolific females and lanes 9-16 represent prolific females.

Thus, the RAPD-PCR technique using the DNA markers was able to separate between prolific and non prolific females in the Damascus breed with high precision. This confirmed the results previously obtained from the same population of goats using the SDS-PAGE technique.

Associations between reproductive and productive traits and blood protein polymorphism (i.e. biochemical genetic markers)

Due to the small number of animals representing, all the calculated values of correlation between the blood protein banding patterns generated by the SDS-PAGE technique and the traits for the Harri and Najdi females were statistically not significant (P>0.05). However, For the non-prolific Harri females (N=8), the correlation values between twinning rate found to be associated negatively and highly significantly (P <0.01) with protein band having molecular weight of 196 KDa, however, it tended to be associated positively with protein bands having molecular weights 43 and 38 KDa and negatively with protein band having molecular weight of 165 KDa. Litter size tended to be associated positively with protein band having molecular weight of 112 KDa and negatively with protein bands having molecular weights 196, 43 and 38 KDa.

For the prolific Harri females (N=8), the correlation values between twinning rate found to be associated positively and significantly (P <0.05) with protein band having molecular weight of 45 KDa, however, it tended to be associated positively with protein band having molecular weight of 67 KDa and negatively with protein bands having molecular weights 130 and 44 KDa. Litter size tended to be associated positively with protein bands having molecular weights 179, 67, 53 and 45 KDa, and negatively with protein band having molecular weight of 152 KDa. For the non-prolific Najdi females (N=8), the correlation values between twinning rate showed statistically non-significant associations (P >0.05) with the different protein bands. Litter size tended to be associated positively with protein band having molecular weight of 42 KDa. Lamping interval tended to be associated positively with protein band having molecular weight of 21 KDa and negatively with protein band having molecular weight of 94 KDa.

For the prolific Najdi females (N=8), the correlation values between twinning rate found to be associated positively and significantly (P <0.05) with protein band having molecular weight of 42 KDa, however, it tended to be associated positively with protein band having molecular weight of 145 KDa and negatively with protein bands having molecular

weights 151 and 44 KDa. Litter size found to be associated positively and significantly (P <0.05) with protein band having molecular weight of 49 KDa, however, it tended to be associated positively with protein band having molecular weight of 42 KDa and negatively with protein band having molecular weight of 151 KDa.

Abdel-Hafez (2002) found association between marker gene alleles and ewes reproductive traits. The alleles Ab1, Ab2, Aa1, Aa2 and A01, A02 of albumin marker gene were associated with the highest values of age at first lambing and lambing interval. The alleles Gc1, Gc2, Aa1, Aa2 and C01, C02 of β-Galactosidas, Albumin and Catalase marker genes were insignificantly associated with the highest values of Kg of lambs produced at weaning /ewe/ season. Marai *et al.* (2001) found associations between reproductive traits of homozygous Nubian doe genotypes and marker gene alleles. The lowest values of age at first kidding were associated with the allele Cb of C. An. Er. They also showed that the highest values of each of litter size and litter weight at birth and at weaning were associated with the allele Ia of Tr.I marker gene and those of kilograms of kids produced / life time of doe at birth and weaning with the allele Aa of A marker gene.

Generally, such positive results may suggest that marker assisted selection could be carried out at early ages for the improvement of reproductive and productive traits in Harri and Najdi sheep. Further studies on the same breeds using more animals, are needed to be carried out to confirm the present results.

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