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SUMMARY

Blood samples were collected from 48 females of different ages taken from the Egyptian Zaraibi goat breed in order to find molecular genetic markers which can differentiate between the prolific and non-prolific females using both RAPD-PCR and SSR techniques. Twenty-six and 22 prolific and non-prolific does, respectively, were chosen from two stations (Sakha and El-Serw) according to the litter size trait.

The results showed that RAPD-PCR technique was precisely able to discriminate between prolific and non-prolific Zaraibi individuals using some specific bands of different molecular weights produced by the selected primers (10 primers). The RAPD-PCR technique was also able to distinguish between the Zaraibi females from Sakha and El-Serw Stations (between animals of the same breed but from different locations).

The results showed also that the SSR technique using the microsatellite markers was able to distinguish between prolific and non-prolific Zaraibi individuals with enough precision by some specific bands produced by 7 microsatellites. Additional markers might be needed in order to complete the genetic characterization and determination of the phylogenetic relationships.

INTRODUCTION

Goat are considered one of the important economic sources of meat in developing countries. Improving the reproductive efficiency of goat herds in these countries, can increase the efficiency of kids production and consequently goat meat. Studies of the inheritance patterns of ovulation rate and litter size in prolific sheep flocks led to segregating a major gene for prolificacy in Booroola sheep (Davis *et al.*, 1982; Piper and Bindon, 1982) and recently in other sheep breeds. Therefore, the identification of the gene responsible for the prolificacy in goat, known as Booroola gene (or FecB gene) in sheep (Piper and Bindon, 1996), is of importance to the goat industry.

On the other hand, the polymorphism of blood genetic markers gives some useful information in studying the relationships among breeds and their evolution. It can also be used for indirect selection if there were some relationships between these markers and some economically important quantitative traits. Many researchers employed the Random amplified polymorphic DNA markers or RAPD technique to characterize and estimate genetic distances between goat breeds (Williams et al., 1990; Welsh and McClelland, 1990; Nyamsamba et al., 2002; Ouafi et al., 2002), in the study of genetic diversity within breeds (Li et al., 1999; El-Seoudy et al., 2005) and in determination of sex and gene mapping in farm animals (genetic linkage maps are now available for goat and sheep; Vaiman et al., 1996).

Molecular methods have also provided new markers such as microsatellites (i.e. highly polymorphic DNA simple sequence repeats; SSRs) for the study of genetic variation and evolutionary relationships of closely related populations (Takezaki and Nei,1996; Diez-Táscon *et al.*, 2000; Moioli *et al.*, 2001; Li *et al.*, 2002; Visser *et al.*, 2004; Iamartino *et al.*, 2005; Kumar *et al.*, 2005).

In Egypt, there are many native goat breeds with different productive and reproductive performances. One of the most important of them is the Zaraibi (or Egyptian Nubian) breed, that reared mainly for milk production. It is characterized by a good prolificacy, i.e. litter size (Marai *et al.*, 2001 and Galal *et al*, 2005). The research to locate the gene responsible for the prolificacy in this breed, using biochemical and molecular genetic markers, may help its use commercially to improve other local non-prolific breeds. The results reported by Marai *et al.* (2001) revealed the existence of association between reproductive traits of Zaraibi (or E. Nubian) doe genotypes and marker gene alleles. Anous *et al.* (2008) studied the genetic relationship among three Egyptian goat breeds using biochemical genetic markers and concluded that molecular techniques at the DNA level will be needed to detect more genetic markers for prolificacy (i.e. litter size) trait.

Therefore, the objective of the present study is to assess the genetic structure within the Egyptian Zaraibi goat population at the DNA level in order to find molecular genetic markers which can differentiate between prolific and non-prolific females and help for the identification of the prolificacy gene in Egyptian goat.

Keywords: Zaraibi goats, Prolificacy, Litter size, Molecular markers, RAPD, SSR.

MATERIAL AND METHODS

I) Animal pedigree records and data collection

The base goat population was assembled in 2006 from two different experimental stations belonging to Animal Production Research Institute (El-Serw and Sakha Stations). A total of 48 does of different ages were collected from the Egyptian Zaraibi goat breed according to their litter size (i.e. prolificacy) trait, using the pedigree records.

II) Animal groups construction and blood samples collection

The individuals of the Zaraibi breed were assembled in two groups; prolific (n = 26) and non-prolific (n = 22) does, according to the prolificacy trait (i.e. number of kids born per parturition per female): where three or more kids per parturition for the first group and one or two kids per parturition for the second group.

Blood samples were collected from the selected females by vacuum glass tubes holding the EDTA disodium salt (EDTA-Na₂) as anticoagulant. Blood serum was then obtained by centrifugation at 5000 rpm for 15 minutes at 4° C and the pellet was stored at -20°C until the time of DNA extraction.

III) Genetic characterization

1. DNA extraction

DNA was isolated with the phenol-chloroform extraction method as described by **Sambrook** *et al.* (1989). Extracted DNA was then quantified using the UV spectrophotometer (Eppendorf BioPhotometer).

2. Random amplified polymorphic DNA (RAPD)

The RAPD-PCR technique was conducted in order to capture population of molecular fingerprints and estimate phylogenetic relationship between the two groups of the Zaraibi females. Initial screening of 10 random RAPD primers (synthesized by Operon, Germany) was undertaken to detect the genetic polymorphisim among and within each one of the two groups of the Egyptian Zaraibi goat breed. The primer manufactured codes, sequences and molecular weights are given in Table 1.

The amplification conditions were carried according to Williams *et al.* (1990). Gel was stained with 0.2 μ g/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.

3. SSR markers

In the present study, initial screening of a total of 7 microsatellite markers out of a list of eighteen microsatellites recommended by the International Society of Animal Genetics (ISAG) for goat diversity studies, was undertaken in order to find molecular genetic markers which can differentiate between the prolific and non-prolific Zaraibi females. They are: ILST019, INRA0005, MAF0065, SRCRSP0005, SRCRSP0024, McM0527 and OarFCB0020. The size of PCR products was determined by electrophoresis in denaturing polyacrylamide gel according to the method described by **Echt** *et al.* (1996). Primers used in the PCR were labeled with a fluorescent tag. Gel analysis was performed with Genescan 672 (Version 1.2) software.

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Primer codes	Primer sequences	Molecular weights (bp)
A14	TCT GTG CTG G	3050
A16	AGC CAG CGA A	3046
A20	GTT GCG ATC C	3019
B08	GTC CAC ACG G	3013
B12	CCT TGA CGC A	2988
C05	GAT GAC CGC C	3013
C06	GAA CGG ACT C	2988
C08	TGG ACC GGT G	3013
C11	AAA GCT GCG G	3037
C20	ACT TCG CCA C	3084

Table 1. List of the studied primers and their sequences and molecular weights.

IV) Statistical analysis

1. Genetic identity and genetic distance

Both genetic identity and distance were estimated according to **Bardacki and Skibinski (1994)**. The banding patterns of individuals were compared within groups. Bands were scored as 1 if present or 0 if absent (i.e. binary values) in order to differentiate between the two groups of Zaraibi females (i.e. prolific and non-prolific females).

2. Dendrogram construction

For constructing a combined Dendrogram for the two groups of the Zaraibi goat breed, the data generated from DNA banding patterns were introduced to SPSS package program according to binary values (1, 0).

RESULTS AND DISCUSSION

I. RAPD polymorphisim analysis

Plates 1-10 and Tables 2 and 3 represent the results of the RAPD fingerprints of individual samples for the two groups of females of the Zaraibi breed (i.e. prolific and non-prolific females) generated by the different primers.

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In general, primers C05 and C11 gave similar results with respect to number of common, polymorphic and monomorphic bands in Zaraibi goat breed followed by primers C06 and C20 (Table 2). However, primers A14, A20 and C08 gave the highest numbers of polymorphic bands (14, 15 and 15, respectively). This means that primers A14, A20 and C08 could be used as markers in order to distinguish between prolific and non-prolific females.

On the other hand, primers C05, C06, C11 and C20 were able to detect five common specific bands with different molecular weights (i.e. 206; 241 and 177; 209; 183 bp) which could be used to characterize the Zaraibi breed as a whole, compared to the other Egyptian goat breeds, as shown in Table (2).

However, primers A20, B12, C05 and C20 showed 1, 2, 1 and 1 specific bands, respectively, at different molecular weights (i.e. 384; 309 and 125; 110; 77 bp) for the prolific females, while primer B08 showed one specific band at molecular weight of 319 bp for the non-prolific females (Table 3). These bands could be used to distinguish between prolific and non-prolific females within the Zaraibi breed. Table (3) showed also the possibility of distinguish between the Zaraibi females of Sakha and El-Serw Stations using some specific bands produced by primers A14, A16, B12 and C06. Thus, the RAPD-PCR technique was also able to differentiate between animals of the same breed but from different locations. This means also that Station might have an effect on the genetic background of Zaraibi females used in this study which may be due to the differences in applied breeding program in the two Stations.

The dendrogram of this population of goat (Figure 1) showed that Zaraibi females were divided into two main groups. Within each group the animals were divided again into two main sub-groups; the smallest one constituted by prolific females from El-Serw Station and non-prolific females from Sakha Station and the biggest one constituted by prolific females from Sakha Station and non-prolific females from El-Serw Station. Thus, the RAPD-PCR technique using the DNA markers was able to separate between prolific and non-prolific individuals of each Station with high precision. This confirmed the results previously obtained with the same sample of goats using the SDS-PAGE technique (Anous *et al.*, 2008). The RAPD-PCR technique was also able to separate between animals of different origin (i.e. stations) from the same breed. These results agreed with those obtained by Abu-Shady (2004) about the genetic variation within and between two Egyptian goat breeds using the RAPD analysis. She reported that there are some bands that differentiate the sex within each breed or location (i.e. station where animals were reared) and other bands that could be used to differentiate the two breeds.

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Table 2. Range of molecular weights (Mw) of bands (bp), range of number of band products and number of bands produced by different primers in Zaraibi goat.

* Values between brackets represent the frequency.

Primers	Range of molecular weights of bands	Range of number of band products	N Com	umber of bands mon Polymorp Monomorphic	hic*
A14	45-2205	0-14		14 (100.0)	
A16	49-1422	0-9		9 (100.0)	
A20	25-1957	0-15		15 (100.0)	
B08	48-595	0-10		10 (100.0)	
B12	92-606	0-9		9 (100.0)	
C05	110-595	015	1	11 (91.6)	1
			(Mw=2	06)	
C06	93-590	2-7	2	5 (71.4)	2
	1.1.1		(Mw=2	41-177)	
C08	78-601	0-15		15 (100.0)	
C11	66-598	1-12	1	11 (91.6)	1
			(Mw=2	09)	
C20	77-507	1-6	1	5 (83.3)	1
		1011022-0	(Mw=1	83)	





Station.





* RAPD fingerprints of individual samples for Zaraibi breed. M = DNA marker; Lanes 1-13 and 14-20 represent, respectively the prolific and non-prolific females of Sakha Station and lanes 21-35 and 36-48 represent, respectively the non-prolific and prolific females of El-Serw Station.

Table 3. Molecular weights of specific bands produced by different primers for both prolific and non-prolific Zaraibi females in Sakha and El-Serw Stations.

		Molecular we	eights (bp)		
Primers Prolific fen		nales	Non-prolific females		
5	Sakha Station	El-Serw Station	Sakha Station	El-Serw Station	
A14	2205-1053-338- 45	211-162 .	1516-707- 238-67	536-431	
A16		128-70-49		293-269	
A20	1957-1006-114 - 25	149	1298-349-56	399-308	
B08	393-83-48	284-196	420-139		
B12	234	264	92	388-316- 199	
C05		383-249- 186		413-222- 161	
C06		266-214		382-299	
C08	309-205-135-78	511-386 - 286	239 -103	490-365	
C11	66	417-86		338-275	
C20					

This also agreed with the conclusion of Li *et al.* (1999) that RAPD technique is very useful in the field of genetic relationship evaluation and that closely related goat populations can be distinguished using such technique.

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II. SSR genetic markers

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Thirty females from the Zaraibi breed were sampled into two groups (i.e. prolific and non-prolific) of equal number (n=15) and analyzed in order to find specific markers characterize each one of the two groups and consequently characterize the females carrying the fecundity gene. The results of their PCR profiles generated by the 7 microsatellite markers are described in Plates 11-17 and Tables 4-6.



Plate (13): Microsatellite SRCRSP0024*

* SSR fingerprints of individual samples for Zaraibi breed. M = DNA marker; Lanes 1-15 represent prolific females and lanes 16-30 represent non-prolific females.





In general, microsatellites MAF0065 and McM0527 gave comparable results with respect to number of common and monomorphic bands (two bands for each) in Zaraibi breed (Table 4) followed by microsatellites INRA0005, SRCRSP0024, ILST019, SRCRSP0005 and

OarFCB0020 (one band for each). However, microsatellites INRA0005 and OarFCB0020 gave the highest numbers of polymorphic bands (2 and 1, respectively). This means that these two microsatellites could be used as markers to characterize the individuals of the two groups of females of such breed. The limited polymorphism obtained in the present study is probably due to the smaller sample size. Barker (1994) reported that the precision of estimated genetic diversity is a function of the number of loci analyzed, the heterozygosity of these loci and the number of animals sampled in each population.

However, microsatellite INRA0005 showed 2 specific bands at different molecular weights (i.e. 184 and 125 bp) for the prolific and non-prolific females, respectively, while microsatellite OarFCB0020 showed another specific band at molecular weight of 071 bp for the prolific females (Table 5). These bands could be used to distinguish between prolific and non-prolific females within the Zaraibi breed.

In conclusion all selected microsatellite metters showed little informative capacities bout the profit is end not-profitic females of the Zaraph bread pacent INRA/00/3 and 0arFCB0020. The INRA/0005 was the most polymorphic, thus the most informative additional markers are being needed in order to complete the genetic characterization of genetic activities of the studying the genetic variation in South African gas Table 4. Range of molecular weights of bands (bp), range of number of band products and number of bands produced by different microsatellites in Zaraibi goat.

	Range of	Range of		Number of bands		
Microsatellites	molecular weights of bands	number of band products	Commo n	Mono- morphic	no- rphic c*	
INRA0005	109-244	0-3	1	1	2 (0.66)	
MAF0065	114-185	2	2	2		
SRCRSP0024	299	1	1	1		
ILST019	134	1	1	1		
SRCRSP0005	340	1	1	1		
OarFCB0020	071-200	0-2	1	1	1 (0.50)	
McM0527	100-327	2	2 .	2	. ,	

* Values between brackets represent the frequency.

Table 5. Molecular weights of specific bands produced by different microsatellites in prolific and non-prolific Zaraibi females.

Microsatellites	Molecular weights (bp)		
	Prolific females	Non-prolific females	
INRA0005	184	125	
OarFCB0020	071		

In conclusion, all selected microsatellite markers showed little informative capacities about the prolific and non-prolific females of the Zaraibi breed except INRA0005 and OarFCB0020. The INRA0005 was the most polymorphic, thus the most informative. Additional markers are being needed in order to complete the genetic characterization of prolificacy in Zaraibi goat. In studying the genetic variation in South African goat

populations using ten microsatellite markers, Visser *et al.* (2004) concluded that additional markers are being tested in order to complete their genetic characterization and determine phylogenetic relationships.

The dendrogram of this population of goat (Figure 2) showed that Zaraibi females were divided into two main groups. The smallest one (situated in the low part of the diagram) is constituted by most of the prolific females and the biggest one (situated in the high part of the diagram) is constituted by most of the non-prolific females. This distribution reflects the degree of homogeneity in this breed. Thus, the SSR technique using the microsatellite markers was able to separate between prolific and non-prolific individuals with high precision. This confirmed the results previously obtained in Zaraibi breed using both the SDS-PAGE technique (Anous *et al.*, 2008) and RAPD technique (present study). This also agreed with the conclusion of both Nyamsamba *et al.* (2002), Li *et al.* (2002), Visser *et al.* (2004), Iamartino *et al.*, 2005 and Kumar *et al.*, 2005 in that closely related goat populations can be distinguished using microsatellite polymorphism data.

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Rescaled Distance Cluster Combine



Figure (2): Average genetic linkage between prolific and non-prolific Zaraibi females.

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التعرف على جين الخصوبة في المعز المصرية باستخدام الكاشفات الوراثية ٢. الكاشفات الجزيئية متعددة المظهر

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نم أخذ عينات دم من عدد 48 أنثى من أعمار مختلفة من سلالة الماعز الزاريبى المصرية وذلك بهدف البحث عن كاشفات وراثية جزيئية تستطيع النفريق بين الإناث عالية الخصب والإناث منخفضة الخصب وذلك باستخدام نوعان من التقنيات الخاصة بالتفريد الكهربى للحمض النووى DNA و هما تقنية RAPD-PCR وتقنية SSR. وقد تم اختيار ٢٦ عنزة عالية الخصب و ٢٢ عنزة منخفضة الخصب من قطيعى محطتى سخا والسرو التابعتين لمعهد بحوث الإنتاج الحيوانى وذلك طبقاً لصفة حجم الخلفة فى البطن الواحد.

وقد أوضحت النتائج أن التفريد الكهربى للحمض النووى DNA باستخدام تقنية RAPD-PCR استطاع أن يفصل بدقة بين إناث الزرايبى عالية الخصب والإناث منخفضة الخصب باستخدام بعض الحزم المحددة ذات الأوزان الجزيئية المختلفة الناتجة عن استخدام عشرة من البادئات والتى يمكن اعتبارها كاشفات جزيئية خاصة لكل مجموعة. وقد استطاع أيضاً استخدام تقنية RAPD-PCR للتمييز بين إناث الزرايبى المرباه في محطة سخا وتلك المرباه في محطة السرو (أى التمييز بين حيوانات من مصادر مختلفة لنفس السلام).

كما أوضحت النتائج أيضا أن تقنية SSR باستخدام بعض الكاشفات الوراثية الجزيئية microsatellite markers استطاعت أن تفصل بدقة كافية بين إناث الزرايبى عالية الخصب والإناث منخفضة الخصب بواسطة بعض الحزم المحددة الناتجة عن استخدام سبعة من الكاشفات الوراثية الجزيئية microsatellite markers. وقد خلصت الدراسة إلى الحاجة إلى كاشفات وراثية جزيئية إضافية لاستكمال التوصيف الوراثي لهاتين المجموعتين ولتحديد العلاقة الوراثية بينهما.