Genetic Differentiation of two Native Egyptian Goat breeds Assessed by Microsatellite DNA Markers

Ghazy, A. *, S. Mokhtar*, Manal Eid*, A. Amin*, M. Elzarei*, K. Kizaki* and K. Hashizume*****

* Animal production Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

** Agricultural Botany Department, Faculty of of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

*** Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka 020-8550, Japan

Received: *24/12/2012*

Abstract: The genomes of two Egyptian native goat breeds were screened using microsatellite markers; the breeds were Zaraibi and Sinai goats. A total of 18 microsatellite markers were used to study the genetic structure and diversity within and between both populations. All eighteen tested loci were polymorphic in both populations. Number of actual alleles (Na), number of effective alleles (Ne), expected heterozygosity (He), polymorphic information content (PIC), Wright's F-statistic values and Nei's standard genetic distance (Ds) were calculated. The overall average number of alleles per locus was 18.4 .Within breeds, the number of alleles ranged from 8 to 26 alleles at the eighteen assessed loci. The average values of Ne, He and PIC of all loci were 12.3, 0.88 and 0.91, respectively. The observed global inbreeding coefficient F_{IS} (0.13) indicated that both goat breeds (Sinai and Zaraibi) are very slightly heading towards inbreeding. Nei's standard genetic distances, yielded relationships between populations that agreed with what is known about their geographical distribution. It was concluded that microsatellite analysis is a useful tool to study the genetic diversity within and between native goat breeds and it can provide basic valuable information to assist in developing a national plan for genetic improvement of indigenous goat breeds in Egypt.

Keywords: genetic diversity, genetic distance, local goat breeds, microsatellite markers.

INTRODUCTION

The Egyptian goats are reported to be domesticated after migration from Asia along the present Iran–Iraq borders and then to Africa (Mason, 1981). In Egypt, there are about 5 million goats (MOLAR, 2005) raised mainly in three regions: the Nile Delta, Upper Egypt and in the desert rangelands. Production systems and breeds in the three zones are different. Zaraibi goats are presented in Northeastern Nile Delta (Galal *et al.*, 2005) and they are the most pronounced dairy goat among the local breeds in Egypt. It is considered of high genetic potential as a dairy and prolific breed (Aboul-Naga *et al.*, 1993), while Sinai goat breed is adapted to arid environment. Throughout their distribution area, water is scarce and the pasture is meager and mostly of low quality-high in fiber and low in protein.

An assessment of genetic variability in domestic goats is the first step towards conservation of genetic resources for maintaining breeding options. Genetic characterization and determination of genetic differences between goat breeds help in the genetic improvement programs. Molecular methods used for detecting molecular markers, such as RAPD, RFLP and microsatellites are useful tools to study the genetic variations. In recent years, microsatellite markers are being used in many studies to determine genetic variability and correlation analysis of economic traits in goats (Jandurova *et al.*, 2004; Jin *et al.*, 2006 and Marrube *et al.*, 2007). Microsatellites have often been used for genetic diversity studies, because of their distribution throughout the genome, high level of polymorphism, co-dominant inheritance and easy to analyze (Cañon *et al.* 2006). In the present study, a set of eighteen microsatellite markers were used to evaluate the genetic diversity within and between two Egyptian goat breeds (Sinai and Zaraibi) and to measure the genetic distance between both breeds.

MATERIALS AND METHODS

Animals and samples collection:

Fifty nine random blood samples were collected from different individuals of two Egyptian goat breeds (Sinai, 31 and Ossimi, 28). Ten ml of blood was collected via the jugular vein in K3EDTA containing tubes for prevention of coagulation. DNA was extracted from blood using the Genomic DNA Purification Kit of (Fermentas Co.). DNA concentration was determined using NanoDrop (Spectrophotometer ND-1000).

Microsatellite analysis:

Eighteen microsatellite markers across the ovine genome were used in the present study. Grading PCR thermal cycle was used to detect the suitable annealing temperature for each marker. The PCR products were tested in agrose gel to estimate the best annealing temperature for each primer. Studied microsatellite markers, their primer sequences, detected annealing temperature and their allele size ranges are shown in Table1.

Statistical analysis:

Genotypes were assigned for each animal based on allele size data. Frequencies and number of alleles for each locus, observed and expected heterozygosity and F-statistics (F_{IS} is the inbreeding coefficient of an individual relative to the subpopulation, F_{IT} is the inbreeding coefficient of an individual relative to the total population and F_{ST} is the effect of subpopulations compared to the total population) were estimated using FSTAT (version 2.9.3.2) (Goudet 2002). The polymorphic information content (PIC) value was calculated according to Botstein *et al.* (1980). Nei's (1987) standard genetic distances (D_S) between both populations were computed by POPGENE (version 1.31) (Yeh *et al.* 1999).

Table (1): Sequences, Chromosome location, annealing temperatures and detected allele size range of microsatellite marker primers.

Microsatellite Names	Primer sequences (5`→ 3`)	Chromosomal location	Annealing temp. °C	Allelic size range (bp)
BM143	F: ACCTGGGAAGCCTCCATATC R: CTGCAGGCAGATTCTTTATCG	6	62	100-120
ETH225	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	9	57	144-184
HSC	F: CTGCCAATGCAGAGACACAAGA R: GTCTGTCTCCTGTCTTGTCATC	20	58.2	264-298
ILSTS005	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	7	55	184-228
ILSTS008	F: GAATCATGGATTTTCTGGGG R: TAGCAGTGAGTGAGGTTGGC	9	51	172-222
ILSTS29	F: TGTTTTGATGGAACACAGCC R: TGGATTTAGACCAGGGTTGG	1	55	146-202
ILSTS30	F: CTGCAGTTCTGCATATGTGG R: CTTAGACAACAGGGGTTTGG	2	55	156-196
ILSTS49	F: CAATTTTCTTGTCTCTCCCC R: GCTGAATCTTGTCAAACAGG	3	51	158-196
ILSTS82	F: TTCGTTCCTCATAGTGCTGG R: AGAGGATTACACCAATCACC	2	55	104-150
ILSTS87	F: AGCAGACATGATGACTCAGC R: CTGCCTCTTTTCTTGAGAGC	6	54	148-180
MAF33	F: GATCTTTGTTTCAATCTATTCCAATTTC R: GATCATCTGAGTGTGAGTATATACAG	9	60	106-130
MAF65	F: AAAGGCCAGAGTATGCAATTAGGAG R: CCACTCCTCCTGAGAATATAACATG	15	60	118-158
MAF70	F: CACGGAGTCACAAAGAGTCAGACC R: GCAGGACTCTACGGGGCCTTTGC	4	63	142-190
OarCP34	F: GCTGAACAATGTGATATGTTCAGG R: GGGACAATACTGTCTTAGATGCTGC	3	53	114-140
OarFCB11	F:GGCCTGAACTCACAAGTTGATATATCTATCAC R: GCAAGCAGGTTCTTTACCACTAGCACC	2	62	130-180
OarFCB20	F: GGAAAACCCCCATATATACCTATAC R: AAATGTGTTTAAGATTCCATACATGTG	2	58	90-128
OarJMP29	F: GTATACACGTGGACACCGCTTTGTAC R: GAAGTGGCAAGATTCAGAGGGGAAG	24	52	114-158
RM004	F: CAGCAAAATATCAGCAAACCT R: CCACCTGGGAAGGCCTTTA	15	55	114-150

The selected microsatellites were amplified with polymorphism chain reaction (PCR) using genomic DNA extracted from individual animals. The PCR was performed for each locus in 10µl reactions consisted of 2µl of Genomic DNA (20ng), 5µl 2X PCR AmpliTag gold PCR Master mix (applied biosystems), 0.4 µl primer mix (50 pmoles) and 2.6 µl DNase free water. The PCR program was carried out at 95°C for 10 min, followed by 35 cycles of 95°C for 30 sec., annealing temperature which was determined for each primer (Table1) for 30 sec. and 72°C for 30 sec., and final extension at 72°C for 10 min. Following the completion of the PCR cycles, 3µl of the reaction products was mixed with 1µl 6X gel loading dye and then loaded into each well of vertical 8% polyacrylamide gel mad with 1X TBE buffer at 100 V for 60 to 90 min and stained with Ethidium bromide (1%). A 50bp DNA ladder was used to estimate allele sizes in base pairs (bp). Figure 1 presents a polyacrylamide gel (8%) (PAGE) for allele concerning BM143 marker as an example of the other eighteen microsatellite markers PCR products.

RESULTS AND DISCUSSIONS

All 18 loci were successfully amplified and a total of 326 alleles were detected, locus ILSTS29 showed the highest number of alleles (26) while BM143 and MAF33 showed the lowest (11) (Table 2) with an overall mean of 18.4 allele. The level of variation depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within the populations (Arora and Bhatia 2006). The number of alleles and effective alleles for each of the eighteen microsatellite markers in both goat breeds is presented in Table 2. The mean number of alleles per locus is 11.1 and 10.3 in Sinai and Zaraibi goats, respectively. The high mean number of alleles in Sinai goats could suggest the existence of heterozygous genotypes in this population.

Generally the mean number of alleles is highly dependent on the sample size because of the unique alleles in populations, which occur at low frequencies and also because of the increased number of observed alleles which depend on the population size. All the 59 individuals in both populations were considered in this study. Effective number of alleles is a measure of allelic evenness. The effective number of alleles is also an index used to reveal the genetic diversity of the

populations. In the present study, the results showed that the total number of effective alleles across breeds ranged from 5.84 for MAF33 to 17.99 for ILSTS29. The effective number of alleles ranged from 5.19 for MAF33 to 16.57 for ILSTS29 in the Sinai goat and from 5.06 for OarJMP29 to 13.89 for ILSTS008 and ILSTS29 in the Zaraibi goat breed (Table 2). The mean of Polymorphic Information Content (PIC) across breeds varied from 0.79 (MAF33) to 0.93 (ILSTS29). The overall mean PIC value of 0.89 reflected the high level of polymorphisms of the used set of microsatellites and heterogeneity of the studied goat populations. However, the high estimates of PIC further substantiated the suitability of the used set of markers to applications in parentage control, linkage-mapping programs in addition to genetic studies in Egyptian goats. All of the markers were highly polymorphic, having PIC values of more than 0.5 as shown in (Botstein *et al.* 1980) Table 2.

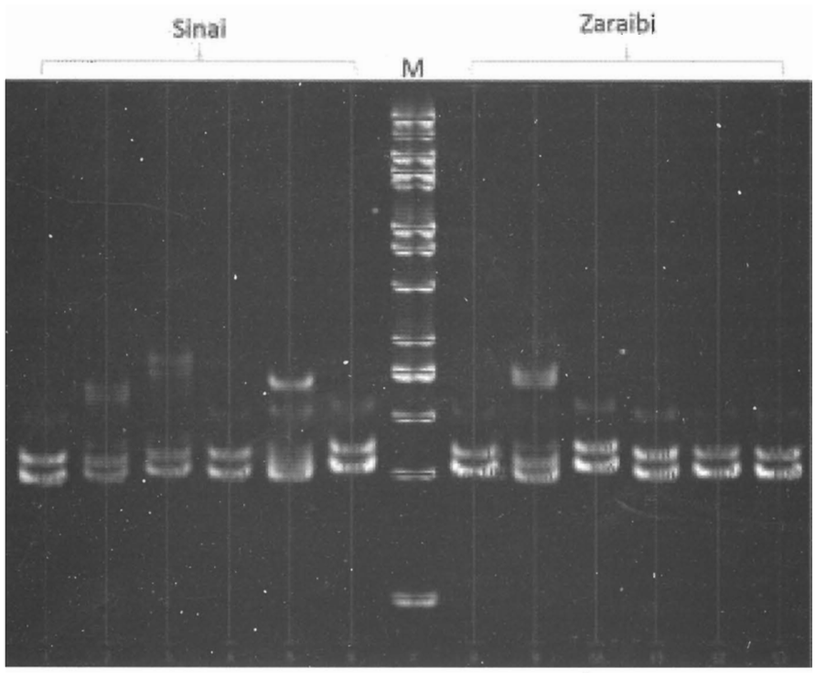

Figure (1): polyacrylamide gel (8%) showing allele concerning BM143 marker. DNA ladder is on well M.

Table (2): Number of observed alleles, number of effective alleles for each locus within breed and Polymorphic Information Content (PIC) in each locus.

Locus	Allelic Number			Effective allelic number			PIC
	Sinai goat	Zaraibi	Total	Sinai goat	Zaraibi	Total	
BM143	11	10	11	8.94	7.61	9.26	0.87
ETH225	16	13	19	9.61	10.38	10.68	0.89
HSC	11	16	18	9.57	10.8	12.12	0.89
ILSTS005	14	13	17	9.2	9.8	10.95	0.89
ILSTS008	19	18	21	13.63	13.89	15.02	0.92
ILSTS29	22	19	26	16.57	13.89	17.99	0.93
ILSTS30	18	18	21	10.68	13.75	14.78	0.91
ILSTS49	16	14	17	12	10.25	12.91	0.90
ILSTS82	16	14	20	10.45	11.36	13.09	0.90
ILSTS87	15	12	16	10.99	8.52	10.73	0.89
MAF33	10	10	11	5.19	5.52	5.84	0.79
MAF65	20	13	21	15.5	10.18	14.79	0.91
MAF70	17	15	21	11.44	10.2	12.91	0.90
OarCP34	13	10	13	8.58	8	8.86	0.87
OarFCB11	21	14	24	14.29	10.57	15.47	0.91
OarFCB20	15	13	20	11.24	9.28	11.78	0.89
OarJMP29	8	9	12	6.67	5.06	6.34	0.80
RM004	12	18	18	8.07	11.79	11.21	0.89
Mean	15.93	13.93	18.40	11.11	10.31	12.36	0.89

The average direct count of heterozygosity (observed heterozygosity) overall loci in Sinai and Zaraibi breeds are 0.797 and 0.803, respectively. Whereas the average expected heterozygosity overall loci in the two breeds are 0.914 and 0.909, respectively (Table 3). The average observed heterozygosity was less than the expected for both breeds which could be due to segregation of non-amplifying (null) alleles and /or selection against heterozygotes or inbreeding (Carmen; 2007). High value of average expected heterozygosity within a breed could be attributed to the large allele numbers detected in the tested loci (Kalinwski 2002). Agha *et al.* (2008) reported that the average genetic variability within the Egyptian goat breeds is relatively high (0.722). Canon *et al.* (2006) estimated the expected heterozygosity within the European and Saudi Arabian goat breeds to be in the range from 0.59 (Orobica) to 0.77 (Abaza). In Indian goat breeds, the observed heterozygosity ranged from 0.16 to 1.00 and the expected heterozygosity varied from 0.19 to 0.90 (Fatima *et al.*, 2008). Differences in heterozygosity values (H_{ob}) observed in the present study and those from other previous works might be ascribed to choice of microsatellite loci type as well as existing population structure.

Heterozygosity deficit within a population is measured by Wright's F_{IS}. The F_{IS} values for both populations are given in Table 4. The mean values of inbreeding coefficients (F_{IS}) for Sinai and Zaraibi were 0.135 and 0.127, respectively. The highest F_{IS} within population was observed for the locus OarJMP29, whereas the lowest values were found for locus ILSTS005 in both populations (Table 4). The negative values of F_{IS} for some loci indicated that the mates were less related to each other in comparison with the average population. The global inbreeding coefficient F_{IS} observed (0.13) in the present study indicate that the goat breeds (Sinai and Zaraibi) are very slightly heading towards inbreeding. Such result is expected because of unplanned and indiscriminate breeding practices. This may be due to the fact that few sires are used for the whole and nearby villages in the breeding region. Agha *et al.* (2008) found that the F_{IS} ranged from 0.091 to 0.168 in Egyptian goat breeds. Fatima *et al.* (2008) reported that the mean values of F_{IS} in three Indian goat breeds ranged from –0.058 to 0.070. In Chinese goat breeds, Li *et al.* (2002) estimated mean F_{IS} value of 0.030 which indicated low level of inbreeding within populations. In order to test genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE), at each locus within and across breeds, results in Table 4 revealed significant departure from HWE ($p<0.001$). Deviation from HWE at microsatellite loci have also been reported in various studies (Barker *et al.* 2001; Laval *et al.* 2000; LuiKart *et al.* 1999; Hassan *et al.* 2003; Agha *et al.* 2008; Fatima *et al.* 2008 and Sadeghi *et al.* 2010). The variation at the microsatellite markers indicated deviations from random mating in the two sampled Egyptian breeds. The mean F_{IT} and F_{ST} values across populations were 0.127 and 0.014, respectively. The low F_{IT} and F_{ST} values which are very close to zero indicated low level of inbreeding in the populations and also refer to low genetic differentiation between populations. The low inbreeding values can be attributed to random mating under field conditions. Genetic differentiations quantified by F_{ST} estimates ranged from 0.007 to 0.019 with a mean of 0.014, indicated that around 1.4% of the total genetic variation was explained by a population difference, and the remaining 98.6% were corresponding to differences among individuals. Agha *et al.* (2008) reported that the F_{ST} estimates ranged from 0.042 between Egyptian Baladi and Barki goat breeds to 0.149 between Zaraibi and one of Italian goat breeds. Furthermore, they suggested that a high genetic similarity among Egyptian goat breeds exists. Similarly, Canon *et al.* (2006) analyzed thirty microsatellite markers in 1426 goats from 45 traditional or rare breeds in 15 European and Middle Eastern countries. In all populations genetic differentiation between breeds was moderate with a mean F_{ST} value of 0.07, but for most (71%) northern and central European breeds, individuals could be assigned to their breeds with a success rate of more than 80%.

Pairwise F_{ST} estimates and Nei's (1987) standard genetic distance between the two tested breeds are also calculated. Pairwise genetic differentiations quantified by F_{ST} estimates indicated that genetic differentiation between Sinai and Zaraibi goat breeds is small (0.0085), reflecting a weak differentiation between these breeds. Agha *et al.* (2008) reported that the F_{ST} estimates ranged from 0.042 between Egyptian Baladi and Barki goat breeds to 0.149 between Zaraibi and one of Italian goat breeds.

Genetic relationship between Sinai and Zaraibi goat populations was estimated using Nei's standard genetic distance. Genetic distance between these two breeds was 0.214. Results indicated that Sinai breed was distinct from the Zaraibi breed. This is expected due to the different geographical distribution of these two breeds. The Sinai goat is a desert breed that lives in the south Sinai region of Egypt, while Zaraibi goat is a Nile valley and Delta breed. Agha *et al.* (2008) estimated the genetic distances between Egyptian Baladi and Zaraibi, Egyptian Baladi and Barki and between Zaraibi and Barki and reported the values 0.163, 0.160 and 0.172, respectively. And the phylogenetic tree showed that the Egyptian Baladi and Zaraibi goats are together, while Barki deviated from the pair. They also suggested that the reason of this result is that Barki goat is a desert breed that lives in the north-western coastal region of Egypt, while both Egyptian Baladi and Zaraibi goats are Nile valley and Delta breeds.

CONCLUSION

This study represents an initial step in investigating the variability at the DNA level within and between two Egyptian goat breeds. The results indicated that both breeds exhibited considerable genetic variation, based on their high mean number of alleles and gene diversity. The results also suggested that the two breeds (Sinai and Zaraibi) are slightly heading towards inbreeding. Sinai breed distributed far away from the Zaraibi breed. The results indicated also that genetic differentiation between Sinai and Zaraibi breed is small, reflecting a

weak differentiation between these breeds.

There is really a need for more thorough analysis of the genetic diversity of local goat breeds from other regions to include more breeds, large sample sizes and additional molecular markers. The evaluation of genetic variations within and between Egyptian goat breeds may be used as basis for the development of a national breeding strategy.

ACKNOWLEDGEMENT

The practical work of this study was supported through a scholarship from Egyptian government and conducted at the Laboratory of Physiology; Department of Veterinary, Faculty of Agriculture, Iwate University-Japan.

Table (3): Mean (M) of heterozygosity in Sinai and Zaraibi goat populations.

Goat population / Heterozygosity	Sinai		Zaraibi		Overall Mean	
	M	SD	M	SD	M	SD
Mean observed heterozygosity	0.797	0.298	0.803	0.032	0.800	0.308
Mean expected heterozygosity	0.914	0.325	0.909	0.034	0.911	0.030

SD: Standard Deviation.

Table (4): F_{IS}, F_{IT} and F_{ST} values and Chi-square (χ^2) test for Hardy Weinberg Equilibrium (HWE) for each locus within and across populations.

Locus	F_{IS}			F_{IT}	F_{ST}	χ^2* (degrees of freedom).
	Sinai	Zaraibi	across breeds			
BM143	0.072	-0.010	0.031	0.029	0.015	200.9* (55)
ETH225	-0.100	-0.089	-0.095	-0.103	0.007	470.1* (171)
HSC	0.234	0.039	0.137	0.135	0.018	438.9* (153)
ILSTS005	-0.106	-0.096	-0.101	-0.100	0.016	256.6* (136)
ILSTS008	-0.063	-0.019	-0.041	-0.051	0.007	395.0* (210)
ILSTS29	0.089	-0.021	0.034	0.028	0.011	459.3* (325)
ILSTS30	0.055	-0.022	0.017	0.016	0.017	299.4* (210)
ILSTS49	-0.038	-0.011	-0.025	-0.027	0.014	279.0* (136)
ILSTS82	0.231	0.117	0.174	0.171	0.017	403.6* (190)
ILSTS87	0.200	0.248	0.224	0.216	0.011	337.2* (120)
MAF33	0.611	0.874	0.743	0.741	0.019	396.6* (55)
MAF65	0.051	0.068	0.060	0.055	0.014	430.6* (210)
MAF70	-0.009	-0.049	-0.029	-0.029	0.016	386.3* (210)
OarCP34	0.103	0.120	0.112	0.102	0.009	242.3* (78)
OarFCB11	0.049	0.037	0.043	0.043	0.018	475.4* (276)
OarFCB20	0.096	0.217	0.157	0.151	0.014	393.6* (190)
OarJMP29	0.926	0.914	0.920	0.919	0.018	691.0* (66)
RM004	0.028	-0.036	-0.004	-0.004	0.017	313.2* (153)
Mean	0.135	0.127	0.131	0.127	0.014	

* $p<0.001$

REFERENCES

Aboul-Naga, A.M.; Aboul-Ela, M.B.; Ibrahim, M.K. and Mousa, M.R. (1993). Estrous performance in Egyptian Nubian (Zaraibi) goats. J. Agric. Sci. Mansoura Univ., 18: 2262-2266.

Agha, S.H.; Pilla, F.; Galal, S.; Shaat, I.; Andrea, M. D.; Reale, S.; Abdelsalam, A.Z.A. and Li, M.H. (2008). Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. J. Anim. Breed. Genet. 125: 194–200.

Arora, R. and Bhatia, S. (2006). Genetic diversity of Magra sheep from India using microsatellite analysis. Asian Australasian J. Animal Sci. 19: 938-942.

Barker, JSF.; Tan, SG.; Moore, SS.; Mukherjee, Tk.; Matheson, JL. and Selvaraj, OS. (2001). Genetic variation within and relationship among populations of Asian goats (Capra hircus). J. Anim. Breed. Genet. 118: 213-233.

Botstein, D.; White, R.L.; Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. Animal J. Hum. Genet. 32: 314–331.

Carmen Maria, (2007). Genetic characterization of indigenous goat populations of Mozambique. Master thesis. Department of Production Animal Studies in Faculty of Veterinary Science, University of Pretoria. South Africa.

Cañon, J.; Garćia, D.; Garćia-Atance, M.A.; Obexer-

Ruff, G.; Lenstra, J.A.; Ajmone-Marsan, P.; Dunner, S. and the Econogene Consortium (2006). Geographical partitioning of goat diversity in Europe and the Middle East. Anim. Genet., 37: 327–334.

Fatima, S.; Bhong, S.D.; Rank, D.N. and Joshi, C.G. (2008). Genetic variability and bottleneck studies in Zalawadi, Gohilwadi and Surti goat breeds of Gujarta (India) using microsatellites. *Small Ruminant Research*. 77: 58-64.

Galal S.; Abdel-Rasoul, F.; Anous, M.R. and Shaat, I. (2005). On-station characterization of small ruminant breeds in Egypt. In: L. Iñiguez (ed.), Characterization of Small Ruminant Breeds in West Asia and North Africa, vol. (2): ICARDA, Aleppo, Syria, 141–193.

Goudet, J. (2002). FSTAT (version 2.9.3.2): a program to Estimate and Test gene Diversities and Fixation Indices. Available from http://www.unil.ch/izea/software/fstat.html. Updated from Goudet (1995).

Hassan, A.A.; AbouMosallam, H.A.; Oraby, H.A.; de Hondt and El-Nahas, S.M. (2003). Genetic Diversity of Three Sheep Breeds in Egypt Based on Microsatellites Analysis. J. Eng. Biotechn. (NRC). 1: 141-150.

Jandurovà, O.M.; Kott, T.; Kottová, B. and Cezrneková, V. (2004). Seven microsatellite markers useful for determining genetic variability in White and Brown Short-Haired goat breeds. Small Rumin. Res., 52: 271–274.

Jin, M.; Guo, C.L.; Hu, J.H.; Gao, W.B. and Wang, W. (2006). Correlation analysis of economic traits in liaoning new breed of cashmere goats using microsatellite DNA markers. Acta Genetica Sinica. 33: 230-235.

Kalinwski, ST. (2002). How many alleles per locus should be used to estimate genetic distances. Heredity. 88: 62-65.

Laval, G.; Iannuccelli, N.; Legault, C.; Milan, D.; Groenen, MAM.; Giuffra, E.; Andersson, L.; Nissen, PH.; Jorgensen, CB.; Beeckmann, P.; Geldermann, H.; Foulley, JL.; Chevalet, C. and Ollivier, L. (2000). Genetic diversity of eleven European pig breeds. Genet. Select. Evolut. 32: 187-203.

Li, J.Y.; Chen, H.; Lan, X.Y.; Kong, X.J. and Min, L.J. (2008). Genetic diversity of five Chinese goat breeds assessed by microsatellite markers. Czech J. Anim. Sci., 53: 315–319.

Luikart, G.; Biju-Duval, MP.; Ertugral, O.; Zagdsuren, Y.; Maudet, C. and Taberlet, P. (1999). Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (Capra hircus). Anim. Genet. 30: 431- 438.

Marrube, G.; Canob, E.M.; Roldan, D.L.; Bidinost, F. ; Abad, M.; Allain, D.; Vaiman, D.; Taddeo, H. and Poli, M. A. (2007). QTL affecting conformation traits in Angora goats. Small Rumin. Res., 71: 255-263.

Mason I.L. (1981). Classification and distribution of goat breeds. In: K. Maijala (ed.), Genetic Resources of Pig, Sheep and Goats. World Animal Science B8. Elsevier, Amsterdam, pp.8.

MOLAR, (2005). Agricultural Statistics, Economic Affairs Sector. Ministry of Agriculture and Land Reclamation, Egypt. pp 233.

Nei, M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York, USA.

Sadeghi Reza; Mahmoudi Bizhan; Bayat Mansour; Jafarzade Ali; Montazeri Gh Sanam; Seyyedreza Seyyedsafavi and Mohammad Nejadgashti (2010). Genetic Diversity of Raeini Goat Population Based on Microsatellite. Journal of Animal and Veterinary Advance. 9: 661-665.

Yeh, F.C.; Yang, R.C. and Boyle, T. (1999). POPGENE (version1.31): A Microsoft Windows-based Freeware for Population Genetic Analysis. University of Alberta and the Centre for International Forestry Research, Edmonton, Canada.