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Original Article

Fish- Mapping and Standard Gtg-Banding Karyotype of Three Egyptian Sheep Breeds

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ABSTRACT

Standardized karyotyping by GTG- banding technique and physical chromosome mapping by Fluorescent *in* Situ Hybridization (FISH) were utilized to characterize the three Egyptian breeds of sheep (Barki, Rahmani and Ossimi). Blood samples were collected from 15 individuals from each breed of sheep. G-banded chromosomes revealed that the karyotype macrostructure was highly conserved and in considerable accordance to the standard karyotype of the *Ovis aries*. The chromosome diploid number was 54 (2n=54, XX / XY). The karyotype formula was 2n, 54 = L^m_6 + M^a_{22} + S^a_{24}+ sex chromosomes. Physical chromosome mapping of the three breeds (Barki, Rahmani and Ossimi) was carried out by localization of two subtelomeric SSR and two (SPRN) related specific sequences. The two subtelomeric SSR sequences revealed six different loci in five chromosomes (1p37, 1p36 and 17q26 with the EPCDV008 probe) and (2q45, 4q22 and 24q24 with the EPCDV016 probe), respectively. In addition, the two (SPRN) related specific sequences were successful in differentiating among the three breeds. The probe OriaBAC273H7 hybridized to a similar locus (20q13) in breeds Rahmani and Ossimi, while, in Barki, it hybridized to a different locus (22q24). However, probe OriaBAC265G4 hybridized to three different loci (17q25, 22q24 and 20q13) in Barki, Rahmani and Ossimi, respectively.

Keywords: Ovis aries, Sheep, Chromosomes, Karyotype, GTG-banding, FISH.

INTRODUCTION

Conservation of animal genetic resources has become an urgent demand nowadays. This is due to the ever increase in human population, the global climate change and the risk of extinction of some valuable genetic resources.

Therefore, conservation of genetic diversity is required to facilitate genetic improvement and selection, and to meet current production needs in various environmental conditions (Glowazki-Mullis *et al.,* 2008 and Mekuriaw *et al.,* 2016). Accurate characterization of livestock breeds is one of the main purposes of germplasm banks. Different methods are available to characterize livestock breeds at the cytogenetic level (Murphy *et al.,* 2004). In addition, the molecular tools have increased the efficiency of germplasm characterization, thus assuring objective criteria for the conservation of genetic resources and establishment of genebanks (Liu *et al.,* 2014). The study of chromosome structure and the precise identification of chromosomes using differential staining techniques such as G- banding and fluorescence *in situ* hybridization (FISH) markers constitute the first step in exploring chromosomal structure. In addition, establishing a standard chromosome nomenclature for each breed, as well as breed characterization at the molecular level, is important for the patent registration of national genetic resources. This information could be extremely useful in supporting breeding programs and would ultimately help to overcome the acute shortage in animal protein (Ali *et al.,* 2011). The molecular techniques have so far been used in the molecular cytogenetic studies of bovid species including the river buffalo (Iannuzzi *et al.,* 2001a and b), cattle (Fronicke and Wienberg, 2001 and Larkin *et al.,* 2003), goat (Di Meo *et al.,* 2003) and sheep (Iannuzzi *et al.,* 2001b and Di Meo *et al.,* 2003 and 2007).

During the last two decades, FISH technique has been widely used in domestic animals for different purposes. This has been applied to identify chromosomal rearrangements, gene mapping, comparative mapping and evolutionary chromosome studies (Farhadi *et al.,* 2013).

In Egypt, sheep represent a valuable source of meat and milk production. There are several indigenous breeds (Othman *et al.,* 2015). The three major Egyptian sheep breeds are Barki, Rahmani and Ossimi, representing 65% of the total sheep population (El Shennawy, 1995). These breeds are reared using minimal resources and are well adapted to local environmental conditions. Therefore, they could have acquired valuable alleles and allelic combinations that could be of importance in animal improvement and breeding programs. It is important to characterize a breed for its conservation (Mahmoudi *et al.,* 2010). Yet, a few cytogenetic and molecular studies on sheep have been undertaken. Therefore, in the present investigation, GTG-banding and FISH markers were used in an attempt to provide fine standard karyotypic details and characterize the main Egyptian breeds of domestic sheep (Barki, Rahmani and Ossimi).

MATERIALS AND METHODS

Samples collection

Forty five peripheral blood samples were collected from three domestic sheep breeds (Barki, Rahmani and Ossimi). Fifteen samples were taken from each breed. In addition. These materials were kindly provided by the Research and Experimental Station, Faculty of Agriculture, Cairo University. Whole blood samples of the three sheep breeds were collected through jugular venipuncture in heparinized and EDTA vacationers. The blood samples were kept at 4ºC and transported to the Cytogenetics and Molecular Genetics Labs at the National Gene Bank, ARC, Egypt.

Karyotype Analysis

Cell culture

The lymphocytes were cultured using the whole blood microculture technique described by Kenthao *et al.,* (2012) with minor modifications.

Unbanded karyotype

Fifteen cells of well-spread mitotic metaphase plates were used for chromosome measurements. The length of short arm (p) and the long arm (q) of each chromosome were measured and the total length (TL) was calculated as (p + q). The relative length (RL %) of each chromosome was estimated in percent of total length of complement according to the formula ((TL/sum TL) x100). While, the formula used for the centromeric index (CI %) was ((p/TL) x100) according to Supanuam *et al.,* (2009) and Ekambaram *et al.,* (2011). CI % was employed to determine the chromosomes type according to Chaiyasut (1989). The numbering and nomenclature of the chromosomes were given as reported by Ford *et al.,* (1980) in the Proceedings of the First International Conference for the Standardisation of Banded Karyotype of Domestic Animals**.**

GTG-banding technique

The GTG-banding technique was applied on the metaphase chromosomes of the sheep breeds under investigation. The GTG-banding technique was adopted from Kenthao *et al.,* (2012). The slides were dried on air and then soaked in working trypsin (0.025% trypsin EDTA) at 37ºC until the termination of trypsin activity (5 to 10 seconds) by washing the slide with phosphate buffer. The trypsinized slides were stained with 20% Giemsa solution for 30 minutes. The numbering of the chromosomes was according to ISCNDA (1989 and 1990), and G-bands was according to Mensher (1987).

Fluorescent *in situ* Hybridization (FISH)

FISH technique was carried out according to Iannuzzi *et al.,* (2013) with some modifications.

Extraction and purification of genomic DNA

The DNA was extracted using the Qiagen Blood and Tissue Kit (cat No.

69506) according to the manufacturer's instructions.

Probes preparation

Total genomic DNA from the blood samples was used as a template to amplify the desired DNA fragments using specific primer pairs. The PCR products were then employed to prepare the probes by labeling using the DIG-Nick- Translation Kit as described by Fujiwara *et al.,* (2007) with some modifications.

Two subtelomeric SSR primer pairs were employed for sheep. In addition, two primer pairs related to the SPRN (gene coding for Shadow of Prion Protein) were used only for sheep. The sequences of the forward and reverse primer pairs were adopted from Vaiman *et al.,* (2000) and Lampo *et al.,* (2007) as shown in Table (1).

Table 1: Names, nucleotide sequences of the primer pairs and annealing temperature (Ta) used for probes preparation

Primer type	Primer name	Forward sequence	Reverse sequence	Ta (°C)	Reference
Subtelomeric SSR primers	EPCDV008	GAC TTT CCA AGA GCT AAG CG (20)	GAT CTC CTC TAA GCT CAC AC (20)	58 °C	Vaiman *et al.* (2000)
	EPCDV0016	CTT CCC GTT CAT GCA TTC TTG (21)	GAG TGT GGT ATC TAA TCC AGC (21)	58 °C	
Specific (SPRN) related primers	OriaBAC273H7	GGG ACC ATC CTG CTG TGA CG (20)	TCC ACT GTC TGC GTC GTC CTC (21)	65 °C	Lampo *et al.* (2007)
	OriaBAC265G4	TGA GAG GTA AGA AGA CCA CCA AA (23)	TCA ACC GCA GAA CTA TGA ACC (21)	63 °C	

Labeling of probes

PCR products of subtelomeric SSRs and specific gene (SPRN) fragments were labelled using the DIG-Nick Translation Mix Kit (Roche, cat No. 11 745 816 910) according to the manufacturer's instructions with minor modifications.

Denaturation and hybridization

The labeled probes were precipitated with ethanol. A probe stock solution was prepared by suspending in hybridization solution. The tubes were incubated for 15 min at 37°C with occasional vortexing until the precipitated DNA dissolved. Then, the probe was diluted from the stock solution to the desired concentration (2 ng/µl) in hybridization solution.

A volume of 20-30µl diluted probe was added to the slide, covered with a cover slip (24x50mm) and sealed with rubber cement. The slides were placed on a hotplate at 80°C for 5 min to denaturize the probe. The slides were hybridized in

a moist chamber at 37°C overnight. The slides hybridized with subtelomeric SSR probes were washed 3 times for 5 min at 37°C with 2×SSC containing 60% formamide, followed by one time for 5 min with the immunological detection buffer. While, the slides hybridized with the specific (SPRN) probes were washed 3 times for 5 min at 45°C with 2×SSC containing 50% formamide, followed by 5 times for 2 min with 2×SSC and one time for 5 min with the detection buffer.

Detection of signals

Hybridization signals were detected using the Anti-Digoxigenin-Rhodamine, Fab fragments Kit (Roche, cat No 11207750910) according to its manual instructions. Stained with 20µl of DAPI (4,6-diamidino- 2-phenylindole) counter stain.

Microscopic examination karyotyping, idiograming and signals imaging

Chromosomes examination and signals imaging were performed using a vertical fluorescence microscope (Leica DM2500) equipped with a cooled monochrome digital camera (Leica DFC340FX). Twenty cells with clearly observable and well spread chromosomes of each male and female sheep were examined and photographed at 100 × magnification under oil immersion. Chromosome counting and karyotyping were performed using the automated karyotyping & FISH software processing (Leica CW4000) system. Idiograms were constructed from complete chromosomes which showed the maximum possible banding patterns in at least ten different metaphase plates.

Hybridization signals were photographed twice at 100 × magnification under oil immersion, first using a blue filter for DAPI and second using a red filter for rhodamine. Thus, chromosome bands showed up in blue after approximately 300 nm exposure, and probes showed up in red and were exposed for approximately 10 seconds. For each probe, eight to ten metaphases were photographed and images were overlapped using automated karyotyping & FISH software processing (Leica CW4000) system.

RESULTS AND DISCUSSION

Karyotype analyses

In the present investigation the karyotype of the three sheep breeds (Barki, Rahmani and Ossimi) were first investigated to assess their correspondence to the standard karyotype of sheep.

Unbanded chromosomes morphological analysis

The three Egyptian breeds of sheep (Barki, Rahmani and Ossimi) belong to the species (*Ovis aries*). This species is characterized by a diploid chromosome number of 54 (Hansen, 1973; Ford *et al.,* 1980; Ansari *et al.,* 1996 and Di Meo *et al.,* 2005). The karyotype is composed of 26 autosomal chromosome pairs. Out of these, 3 pairs are metacentric while, 23 pairs are acrocentric (Ahmad and Khan,

2007; Ali *et al.,* 2011 and Pinthon and Pomthong, 2011). While, some discrepancies about the type of the X and Y chromosomes were reported in the literature (Makino *et al.,* 1967; Hansen, 1973; Di Meo *et al.,* 2005; Ali *et al.,* 2011 and Arslan and Zima, 2011). In the present investigation the karyotype was based on the analysis of twenty well spread metaphase cells of each male and female sheep **(**Fig. 1). The relative length (RL %), centromeric index (CI %) and centromere position were estimated as displayed in Table (2). In general, the morphological features of the chromosome complement of the three sheep breeds were in accordance with the basic sheep karyotype revealing the karyotype formula 2n = 54 = L^m+ M^a_{22}+ S^a24+ sex chromosomes. For each chromosome pair, slight differences in the measured parameters were detected among the three breeds as shown in Table (2), and illustrated in the histograms (Figs. 2 and 3). The relative length of the 26 autosomal chromosomes across the three breeds ranged from 8.31% (for chromosome 1) to 1.95% (for chromosome 26). The relative length of the longest chromosome (chromosome 1) in Barki, Rahmani and Ossimi breeds was 8.33, 8.31 and 8.33%, respectively. While, the shortest autosomal chromosome (chromosome 26) revealed a relative length of 1.95, 1.96 and 1.97% in Barki, Rahmani and Ossimi, respectively. Similarly, the three breeds revealed negligible differences in the relative length of the X (4.44, 4.46 and 4.47%) and Y (1.43, 1.43 and 1.47%) chromosomes in Barki, Rahmani and Ossimi sheep breeds, respectively. These slight differences could be attributed to different levels of chromosome condensation among the three breeds. These results are in agreement with Ansari *et al.,* (1996) and Di Meo *et al.,* (2005). The centromeric indices for the chromosomes of the three sheep breeds are presented in Table (2) and illustrated as histogram in (Fig. 3). The results confirmed the metacentric nature of the three longest chromosomes. The centromeric index for the longest chromosome (chromosome1) was 47.7, 45.7 and 45.4% in Barki, Rahmani and Ossimi breeds, respectively. While, this index was 45.5, 46.3 and 45.5 for chromosome 2 and 48.6, 48.1 and 47.4 for chromosome 3 in the three breeds. The rest of the autosomes were acrocentric with 0.0% centromeric index in the three breeds. In addition, the X chromosome proved to be submetacentric with CI% of 30.3, 29.4 and 29.8% for Barki, Rahmani and Ossimi breeds, respectively and the Y chromosome was acrocentric. These findings are in agreement with several investigations (Makino *et al.,* 1967; Hansen, 1973 and Di Meo *et al.,* 2005). While, Melander (1959) mentioned that the Y chromosome has a median centromere. In addition, Ali *et al.,* (2011) reported that in Lohi sheep breed, the X chromosome was the largest sub-acrocentric with minute p arm extensions, whereas the Y chromosome

was observed as an asterisk-shaped metacentric. Moreover, Arslan and Zima (2011) reported that in Konya wild sheep, the X chromosome was the longest acrocentric with a distinct short arm, whereas the Y chromosome was the smallest element and metacentric.

Table 2: Relative length (RL %), centromeric index (CI %) and centromere position (CP) of each chromosome for the three sheep breeds (Barki, Rahmani and Ossimi).

Chr. No.	Relative length (RL %) of the chromosomes			Centromeric index (CI) of the chromosomes			Centromere position (CP)
	Barki	Rahmani	Ossimi	Barki	Rahmani	Ossimi	
1	8.33	8.31	8.33	47.7	45.7	45.4	metacentric
2	7.57	7.47	7.43	45.5	46.3	45.5	metacentric
3	7.20	7.16	7.20	48.6	48.1	47.4	metacentric
4	4.56	4.54	4.53	0.0	0.0	0.0	acrocentric
5	4.26	4.26	4.20	0.0	0.0	0.0	acrocentric
6	3.96	4.06	3.99	0.0	0.0	0.0	acrocentric
7	3.84	3.85	3.87	0.0	0.0	0.0	acrocentric
8	3.77	3.75	3.76	0.0	0.0	0.0	acrocentric
9	3.60	3.65	3.60	0.0	0.0	0.0	acrocentric
10	3.53	3.53	3.54	0.0	0.0	0.0	acrocentric
11	3.44	3.46	3.42	0.0	0.0	0.0	acrocentric
12	3.34	3.36	3.39	0.0	0.0	0.0	acrocentric
13	3.31	3.30	3.29	0.0	0.0	0.0	acrocentric
14	3.14	3.16	3.16	0.0	0.0	0.0	acrocentric
15	3.00	3.02	3.04	0.0	0.0	0.0	acrocentric
16	2.97	2.93	2.95	0.0	0.0	0.0	acrocentric
17	2.88	2.83	2.89	0.0	0.0	0.0	acrocentric
18	2.81	2.71	2.78	0.0	0.0	0.0	acrocentric
19	2.66	2.61	2.68	0.0	0.0	0.0	acrocentric
20	2.58	2.52	2.57	0.0	0.0	0.0	acrocentric
21	2.46	2.46	2.49	0.0	0.0	0.0	acrocentric
22	2.41	2.40	2.39	0.0	0.0	0.0	acrocentric
23	2.32	2.32	2.34	0.0	0.0	0.0	acrocentric
24	2.17	2.19	2.21	0.0	0.0	0.0	acrocentric
25	2.08	2.10	2.09	0.0	0.0	0.0	acrocentric
26	1.95	1.96	1.97	0.0	0.0	0.0	acrocentric
X	4.44	4.46	4.47	30.3	29.4	29.8	submetacentric
Y	1.43	1.43	1.47	0.0	0.0	0.0	acrocentric

This examination of the three Egyptian sheep breeds revealed that the fundamental number (NF, number of chromosome arms) of the sheep was NFa=58 for the autosomal chromosomes while, the total was NF= 61 in the male and NF=62 in the female. Similar NFa value of the sheep autosomal chromosomes (NFa=58) was reported by Arslan and Zima (2011). In this respect, Arslan and Zima (2011) reported that, the family Bovidae includes several species

demonstrating variable diploid chromosome numbers but having similar fundamental numbers (NF=60), which, with the exception of a few cases, vary between 58 and 62. The karyotype contains variable numbers of centric fusions, or Robertsonian translocations, which have changed the diploid number but not the NF. The variation in the morphology of the sex chromosomes between the present study and the findings reported by Nicodemo *et al.,* (2008), Xing-tang *et al.,* (2008) and Pinthong and Pomthong (2011), could be attributed to the different origin of the studied animals and/or structural chromosomal rearrangements.

GTG-banding analysis

Cytological examination of the G-banded chromosomes for the three Egyptian sheep breeds (Barki, Rahmani and Ossimi) revealed that the karyotype macrostructures were highly conserved and in general, in considerable accordance to the standard karyotype of the sheep (*Ovis aries*) presented by Mensher (1987) and published by the Committee for Standardized Karyotype of *Ovis aries* (1985).

The G-banding method using the photolytic enzyme trypsin affects the interaction that stabilises the structure of different proteins and nucleic acid components of the chromatin. Therefore, the G-band mechanism is based mainly upon differences in protein composition and organization (Holmquit, 1988 and Ali *et al.,* 2011). It has also been suggested that trypsin treatment leads to the unfolding of protein loops and permits the protein structure associated with the alignment of AT-rich sequences, as reported by Popescu *et al.,* (2000).

The results obtained by Ali *et al.,* (2011) on Lohi sheep were highly inconsistent regarding standard trypsin exposure time for the satisfactory induction of G-bands, which made precise identification of individual chromosomal bands challenging. According to Wiscovitch *et al.,* (1974) and Zhuang *et al., (*2006) this may have been due to relative humidity, room temperature, and the variable extent of slide maturity during overnight incubation. Therefore, in the present study the optimization of trypsinisation was conducted. The concentration and time for trypsin treatment was (0.025% trypsin EDTA) and (5 to 10 seconds) at 37^{o}C. This facilitated the accurate identification of individual chromosomal bands.

Moreover, examination of the G-banded metaphase cells revealed variation in the number of bands in each chromosome depending on the degree of the chromosome contraction. Therefore, the analysis of G-banding has been conducted on early metaphase chromosomes as they displayed the highest number of G-bands and thus had the greatest utility for detailed comparative analysis.

The G-band idiogram of the chromosomes was developed based on

twenty selected metaphases using automated karyotyping & FISH software processing (Leica CW4000).

GTG- banding karyotype of sheep (*Ovis aries*) confirmed that, the chromosome diploid number was 54 (2n=54, XX/ XY) and the karyotype formula was 2n=54 = $L^m + M^a_{22} + S^a_{24}$+ sex chromosomes (Figs. 4 and 5). The G-banded idiogram of each of the three Egyptian sheep breeds consisted of 345 bands in one set of haploid chromosome complement, including sex chromosomes. The number of G-positive (heterochromatic) bands was 159, whereas the total number of G-negative (euchromatic) bands was 186 (Table 3).

Table 3: Subtelomeric SSR probes, specific SPRN related probes and their hybridized loci in the chromosomes of the three sheep breeds.

Probe type	Probe name	Loci in			Loci in references
		Barki	Rahmani	Ossimi	
Subtelomeric SSR probes	EPCDV008	1p37	1p36	17q26	1p36-37 and 17q26 (Vaiman *et al.*, 2000)
	EPCDV016	2q45	4q22*	24q24	2q45, 24q24 and 4q22 (Vaiman *et al.*, 2000)
Specific SPRN related probes	OriaBAC273H7	22q24	20q13	20q13	22q24 (Lampo *et al.,* 2007)
	OriaBAC265G4	17q25	22q24	20q13	

* Nontelomeric locus

At the cytogenetic level, the identification of chromosomes 4, 5 and 6 was difficult, due to their indistinct band organization. Similarly, chromosomes 8, 9 and 10 were often difficult to differentiate. The precise identification of chromosomes 19 to 26 also required great care due to their small size. These results are in agreement with Ansari *et al.,* (1999), Lopez and Arruga (1996) and Stone and Stephens (1993).

The G- banded idiogram of the three Egyptian sheep breeds could be identified as four chromosomal groups.

The first group was consisted of three large metacentric pairs, chromosomes no. 1, 2, and 3. In this group the G-banding pattern revealed three arms (1p, 1q and 2q) with two regions, while, in the other three arms (2p, 3p and 3q) the G-bands constituted one region. The number of bands in the chromosomes 1, 2 and 3 was 30, 34 and 26, respectively. The percentage of band length to the total length of the chromosome ranged from 1.3 for the dark band 1q21 to 15.87 for the light band 3p13. While, the relative lengths of these three chromosomes to the chromosome complement were 9.8, 9.32 and 7.81 for chromosome 1, 2, and 3, respectively.

The second group was consisted of eleven medium acrocentric pairs, from chromosome no. (4) to chromosome no. (14). The G-banding pattern of these eleven chromosomes was comprised of one region. Fifteen bands were observed in the three chromosomes no. 4, 5 and 8,

thirteen bands were detected in the chromosomes no. 11 and 13, and also twelve bands were observed in the chromosomes no. 7 and 9. While, the number of bands in the four chromosomes no. 6, 10, 12 and 14 were 14, 11, 10 and 9, respectively. The percentage of band length to the total length of the chromosome ranged from 1.43 observed as a light band in chromosome 4 (4q111) to 25.00 for the light band 9q13. While, the relative length of this group of chromosomes ranged from 3.0 for chromosome 14 to 4.0 for chromosome 4.

The third group was consisted of twelve small acrocentric pairs, from chromosome no. (15) to chromosome no. (26), where all the bands were in one region. Eleven bands were detected in two chromosomes no. 15 and 17, nine bands were observed in the five chromosomes no. 16, 18, 20, 24, and 25, seven bands were observed in two chromosomes no. 19 and 21, and five bands were observed in two chromosomes no. 22 and 26. While, six bands were detected in the chromosome no. 23. The percentage of band length to the total length of the chromosome ranged from 2.86 for the light band observed in chromosome 20 (20q17) to 27.27 for the dark band in chromosome 22 (22q12). While, the relative length of this group of chromosomes ranged from 2.02 for chromosome 26 to 2.89 for chromosome 15.

The fourth group comprised the two sex chromosomes (X and Y). The X chromosome was submetacentric where the long arm (q) was divided into two G-banded regions, while, the short arm had one region. The short arm comprised three bands, while, in the long arm, eleven bands were present in the two regions. The percentage of band length to the total length of the chromosome X ranged from 1.96 for the light band (Xq13) to 57.89 for the dark band (Xp12). The acrocentric Y chromosome comprised five bands represented in one region. The percentage of band length to the total length of the chromosome Y ranged from 12.50 for the light band (Yq15) to 31.25 for the light band (Yq11). While, the relative lengths of the X and Y chromosomes were 5.42 and 1.43, respectively.

Fluorescent in *Situ* Hybridization (FISH) analysis

Molecular techniques including Fluorescent in *Situ* Hybridization (FISH) have been used for chromosome studies and breed characterization in several mammalian species such as river buffalo, cattle, sheep and goat **(**Pauciullo *et al.,* 2014, Di Meo *et al.,* 2007 and Iannuzzi *et al.,* 2013). This technique enables the physical localization of one or more probes along the chromosomes with high accuracy.

In the present investigation two subtelomeric microsatellite probes (EPCDV008 and EPCDV016) and two specific SPRN related probes (OriaBAC273H7 and OriaBAC265G4) were hybridized to the metaphase

chromosomes. The use of DAPI-stained chromosomes with the rhodamine-labeled probes was useful to accurately assigning each of the probes to its cytological location. Polymorphic hybridization sites were observed among the investigated breeds.

In chromosome spreads with telomeric signals, two signals were visible with TRITC -filter as two spots at both ends of each chromosome, regardless of the age of the slides. However, there were variations in signal intensities among chromosomes, which appeared to be random. Across the three sheep breeds, the four probes hybridized to a total of nine different loci in seven autosomal chromosomes (No. 1, 2, 4, 17, 20, 22 and 24) (Table 3).

Microsatellites subtelomeric probes (EPCDV008 and EPCDV016) hybridized to six different loci distributed in five chromosomes (No. 1, 2, 4, 17 and 24). Five loci were located in the subtelomeric region of chromosomes (No. 1, 2, 17 and 24). While, only one non-subtelomeric locus was hybridized with EPCDV016 in Rahmani sheep. This was located on chromosome No.4 (4q22) as an interstitial band**.**

The EPCDV008 probe hybridized to the short arm (p) of chromosomes No.1 in Barki and Rahmani breeds (1p37 and 1p36) **(**Fig. 6) and to chromosome No.17 (17q26) (Fig.6) in the Ossimi breed. These three loci were reported by Vaiman *et al.,* (2000) for the same probe in sheep. While, the EPCDV016 probe revealed three different loci, two subtelomeric loci in Barki and Ossimi (2q45 and 24q24) (Fig.7), and a unique interstitial band on chromosome No.4 (4q22) (Fig.7) in Rahmani. These results are in good agreement with Vaiman *et al.,* (2000). Therefore, the results proved that the two microsatellites subtelomeric probes (EPCDV008 and EPCDV016) were successful in differentiating among the three breeds.

In situ hybridization of the specific SPRN related probes (OriaBAC273H7 and OriaBAC265G4) with the three sheep breeds revealed three different loci on three small acrocentric chromosomes (No. 17, 20 and 22). The signals were visible as two spots on each chromosome, except in the sheep Barki where only one spot was detected per chromosome. Similarly, Sera *et al.*, (1995) found one spot at each end of the chromosome when hybridizing the sheep chromosomes with the telomeric (TTAGGG) sequence. The probe OriaBAC273H7 hybridized to a similar locus (20q13) in breeds Rahmani and Ossimi (Fig.8), while, in Barki, it hybridized to a different locus (22q24) (Fig.8). However, probe OriaBAC265G4 hybridized to three different loci (17q25, 22q24 and 20q13) in Barki, Rahmani and Ossimi, (Fig.9). This demonstrates that the probe OriaBAC265G4 was successful in differentiating among the three breeds, while, OriaBAC273H7 was only successful in characterizing the Barki breed**.** In this context Lampo *et al.,* (2007) localized the two probes (OriaBAC273H7 and OriaBAC265G4)

on the 22q24 locus in sheep. Therefore, the present results are not in complete accordance with those of Lampo *et al.,* (2007). This could be attributed to the different breeds investigated in both studies.

The present results assured that the FISH-mapping technique is a powerful tool in cytogenetic investigations. In this context, FISH has been reported previously as the best and fast method to (a) physically map loci in specific chromosome regions (Di Meo *et al.,* 2007 and Goldammer *et al.,* 2009), (b) identify correctly chromosomes and chromosome regions involved in chromosome abnormalities (Iannuzzi *et al.,* 2001a and Molteni *et al.,* 2007), (c) anchor radiation hybrid maps to specific chromosome regions (Perucatti *et al.,* 2009) and (d) clarify the chromosome evolution of species by analyzing the gene order among homologous chromosomes of species (Iannuzzi *et al.,* 2009).

It is worth noting that the variation observed in FISH analysis among the three sheep breeds could be due to their different origin and / or the occurrence of rearrangements in their genomes. The sheep Barki breed originated in North Africa in the Coastal Mediterranean Zone. While, the origin of the Ossimi breed is Giza and it is the most popular among the Nile Valley and Delta breeds. Rahmani originated in Northern Syria and Southern Turkey and was introduced into Egypt in the 19th century **(**El Shennawy, 1995).

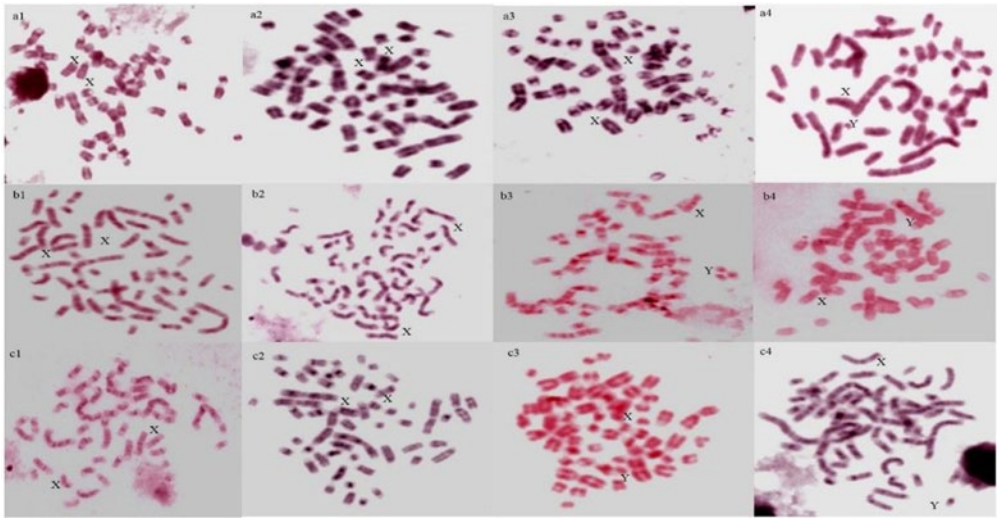

Fig. 1: Spread metaphase cells of male and female for the three sheep, (a1, a2 and a3) female Barki, (a4) male Barki, (b1and b2) female Rahmani, (b3and b4) male Rahmani, (c1and c2) female Ossimi and (c3and c4) male Ossimi

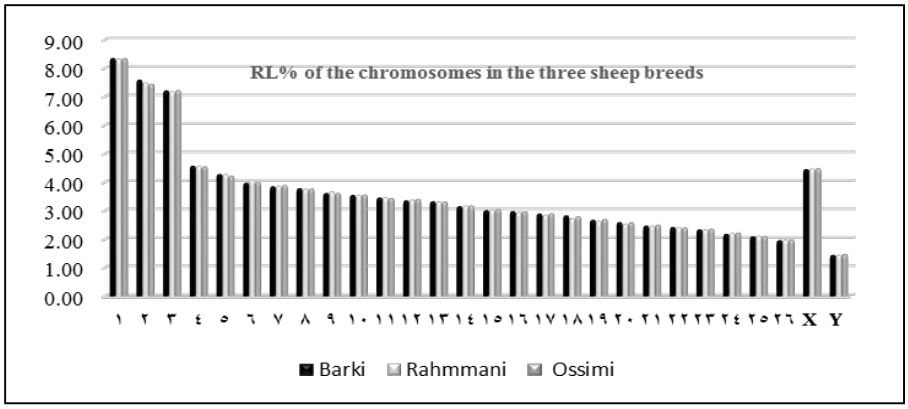

Fig.2:-Histogram showing the relative length (RL %) of the 26 autosomal pairs and the X and Y chromosomes in the three sheep breeds (Barki, Rahmani and Ossimi)

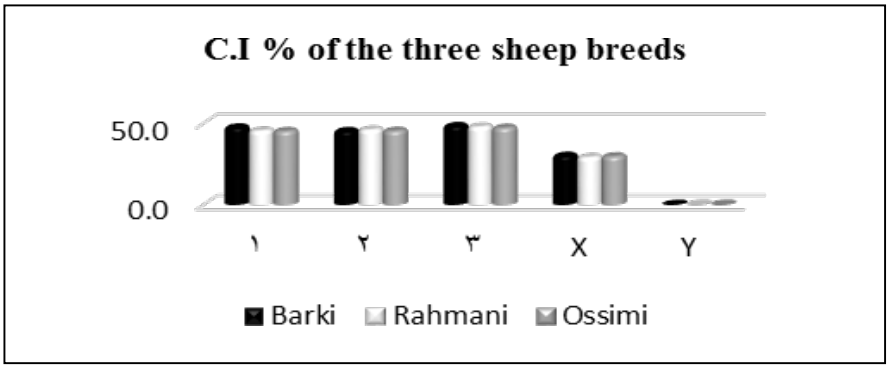

Fig 3: Histogram showing the centromeric index (CI %) of the three sheep breeds: Barki, Rahmani and Ossimi for the chromosomes 1, 2, 3, X and Y

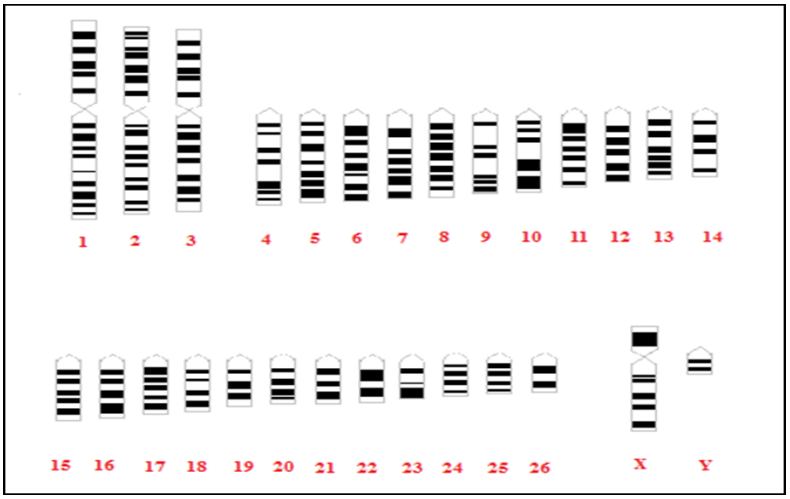

Fig 4: Idiogram of sheep (*Ovis aries*) 2n= 54 as revealed by the G- banding technique

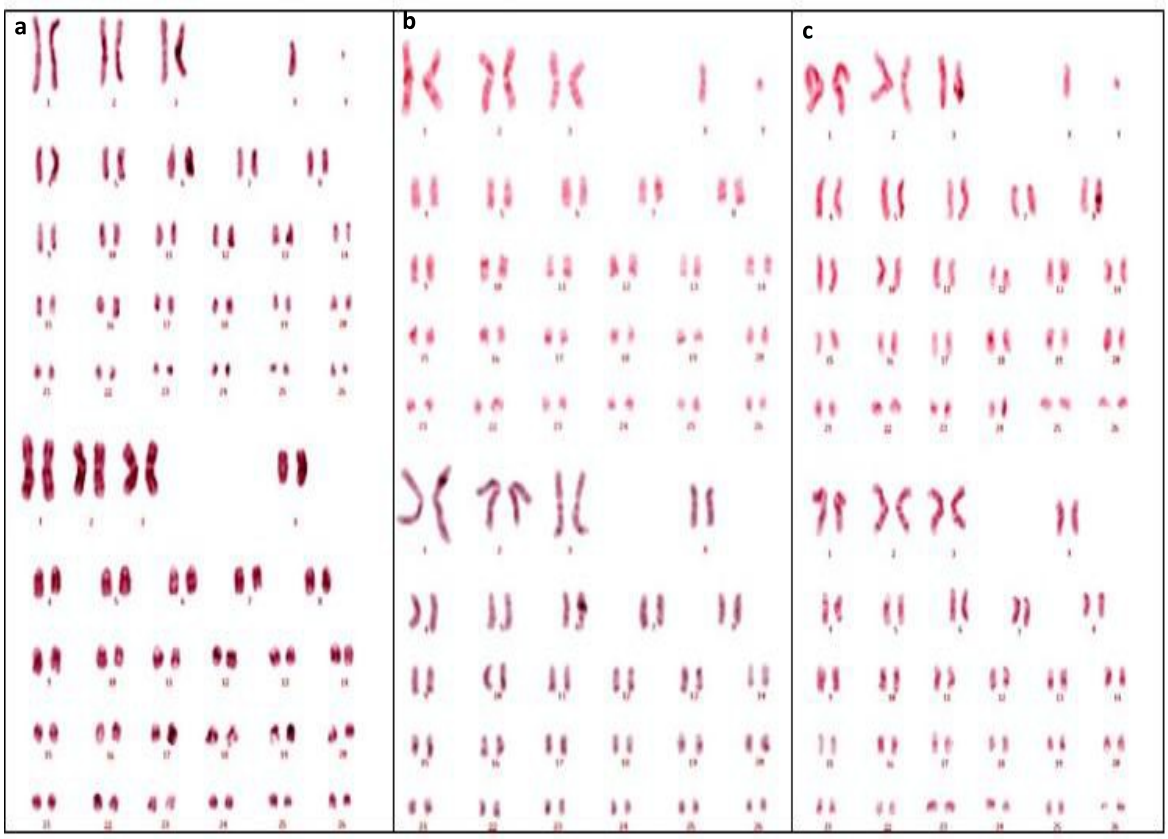

Fig 5: GTG-banding karyotype of male and female of Barki, Rahmani and Ossimi breeds (a, b and c) respectively.

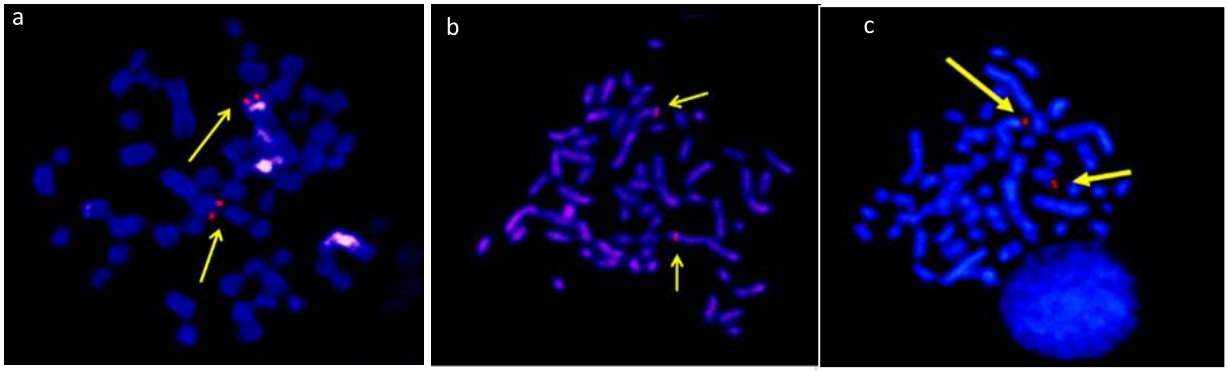

Fig 6: FISH-mapping of subtelomeric SSR Dig-probe (EPCDV008) with the sheep breeds. (a) Barki, in locus (1p37). (b) Rahmani in locus (1p36). (c) Ossimi, in locus (17q26) with metaphase with mixed filter.

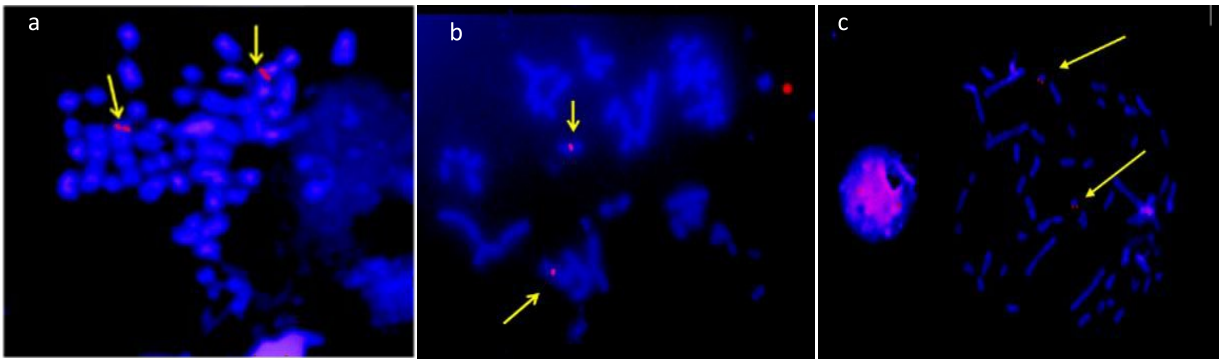

Fig7: FISH-mapping of subtelomeric SSR Dig-probe (EPCDV016) with the sheep breeds. (a) Barki, in locus (2q45). (b) Rahmani , in locus (4q22). (c) Ossimi, in locus (24q24) with metaphase with mixed filter.

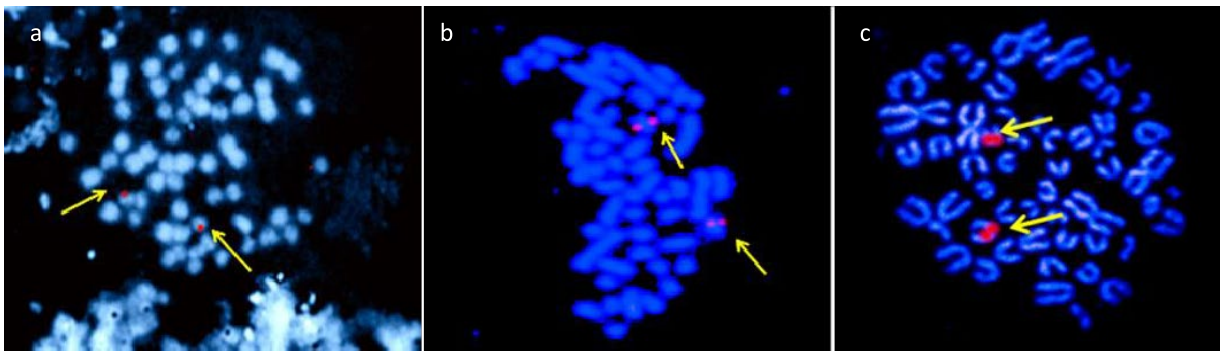

Fig 8: FISH-mapping of the specific SPRN related Dig-probe (BAC273H7) with the sheep breeds. (a) Barki, in locus (22q24). (b) Rahmani , in locus (20q13). (c) Ossimi, in locus (20q13) with metaphase with mixed filter.

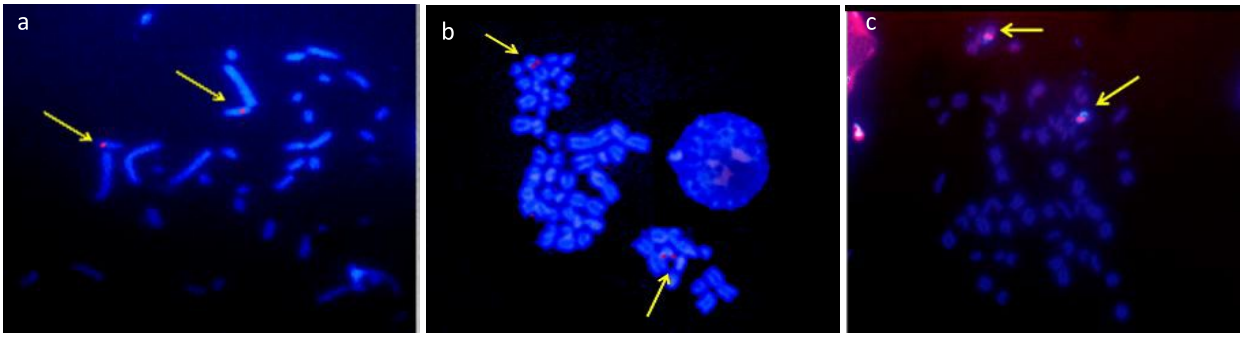

Fig. 9: FISH-mapping of the specific SPRN related Dig-probe (BAC265G4) with the sheep breeds (a) Barki, in locus (17q25). (b) Rahmani , in locus (22q24).(c) Ossimi, in locus (20q13). with metaphase with mixed filter

CONCLUSION

In conclusion, in the present cytogenetic study the first standard karyotype for the three main Egyptian sheep breeds was established by GTG-banding. In addition, the results revealed the adequacy of the FISH technique for physically mapping two subtelomeric and two specific SPRN related loci to the Egyptian sheep breed chromosomes. All hybridization signals

were different, indicating high specificity and reproducibility of the technique used and at the same time proving that these subtelomeric and specific SPRN related loci are suitable markers for accurate identification of Egyptian breed chromosomes or specific regions within them.

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