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THE IDENTIFICATION OF CHROMOSOMAL POLYMORPHISM IN ONE BREED OF IRAQI SHEEP

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SUMMARY

A chromosomal analysis of three breeds of Iraqi sheep (Karadi Arabi and Awassi) has been performed on peripheral lymphocytes. The modal chromosome number of all the three breeds was found to be (2n=54XX/54XY) for femal and male respectively.
The karyotype of Awassi differ from the rest of the domestic sheep (Ovis aries) by the presence of a pericentric inversion on one copy of chromosome number 3.

Basal minimal medium supplemented with foetal bovine serum was the most suitable culture medium to produce sufficient mitotic cells for chromosomal analysis.

INTRODUCTION

Chromosome polymorphism defined as the presence of at least two variants of a given chromosome each with appreciable frequency. Many species have been reported that show a chromosomal polymorphism e.g. Nucella Lapillus (1,2). In comparison with Nucella, no inversion polymorphism has been reported neither in domestic sheep nor in farm animals. However, high frequency of centric fusion (Robertsonian translocation) has been described in several species of domestic animals (3) and in Romney marsh sheep of England (4) but it is generaly believed

that their formation does not mean that significant genetic material is lost. The aim of the present study was to examine the effect of geographical distribution of Iraqi domestic sheep on chromosome morphology.

MATERIAL AND METHODS

A total of 150 sheep (50 individual from each breed) were subjected to the present investigation.

a-Microculture of Leucocytes:

Blood culture was carried out by a modification of the method of moorhead et al.(5).

About 5ml. of blood drawn in to a heparinised (coated) syringe. The blood is immediatly extruded into a culture test tube containing 4ml. of Eagle basal medium (BME), 1ml. foetal bovine serum, 0.25 phytohaemagglutinin (PHA). The PH of the culture medium was adjusted by adding few drops of 7.5% sodium bicarbonate. About 0.2-0.4 ml. of blood was added per 5ml. culture and incubated at 37 °C for 96 hours. Each was shaken twice daily.

b-Chromosome Cytology:

Colchicine was added at a final concentration of 0.004% (W/V) and incubation continued for further 4 hours. At the end of the incubation period, the contents of the cultured bottles were transferred to conical 15ml. centrifuge tubes and centrifuged at 150g for 5 minutes at room temperature. The supernatent was discarded and the pelleted cells gently resuspended in the remaining media. Approximately 2ml of pre-warmed 0.075m KCl was added and the suspension was incubated in a waterbath at 37 °C for 30 minutes. After centrifugation the supernatent was discarded and the cells were re-suspended as above.Two ml of freshly prepared chilled fixative (Methanol: Glacial Acetic Acid) (3:1 V/V) was added dropwise with constant agitation and the tubes were then placed on ice for 15 minutes. The fixative was changed twice and finally the contents suspended in about 0.3ml. of fixative. The cell supension was added dropwise using a pasteur pipatte to cold wet slides. The slides were dried in a stream of

cold air and stored in dust proof boxes at room temperature

The slides were cleaned before use by boiling in a distilled water for 30 minutes and rinsed quickly in a double distilled water.
They were then stored at 4 °C in a double distilled water.

c-Giemsa Staining Technique:

Air-dried slides were placed horizentally on staining rackes and flooded with 10% (V/V) Giemsa staine in 0.067M sorrensons buffer (PH 6.8) for 10 minutes followed by a brief washing in running tap water. The slides were then mounted in dePeX after air drying.

RESULTS

a-Chromosome Analysis:

The cytogenic studies of the three different breeds are shown in table-1.

The chromosome complements of all the three breeds were 2n= 54XX for all the ewes and 54Xy for all the rams. It is noticeable that in all the three breeds, the three largest chromosomes are metacentric chromosome with centromeric index (CI) ranging from 41.6-50. One copy from the third pair of Awassi breed, however, is of submetacentric appearance and can be recognised easily from the rest of the complement with a centromeric index (CI) of 28.1 (Table-2).
For each species studied a representitive metaphase is displayed (Figs. 1,2,3).
The magnification in all photographes is X2500.

b-Leucocyte culture Technique:

The leucocyte culture was developed using blood withdrawn from the jugular vein. Table 3 gives details of the relative success of various conditions. It is clear that the number of chromosome spreads is proportional to the concentration of phytohemagglutinin (PHA) present. The optimal medium appear to be BME plus bovine serum. It was subsequently noted that peak mitotic activity from stimulated lymphocytes occured after 96 hours rather than

Table 1: List of the breeds investigated cytogenetically

Breed	Location	No.of sheep investigated Cytogenetically	Phenotype	Fertility	% of population	Body weight
Arabi	South of Iraq	25 males+25 females	different coulor wool,the ram with a horn,the ewe without born	fair	20%	ram 55kg ewe 45kg
Karadi	north of Iraq	25 males+25 females	Black face and white body,no horn in both sexs	V.good	20%	ram 80kg ewe 65kg
Awassi	middle of Iraq	25 males+25 females	Red or brown face and White body,the ram with horn and big nose, the ewe without horn at all	good	55%	ram 65kg ewe 50kg

Table 2: The chromosome designation and centromeric index of the first three chromosomes

Breed	Chromosome No.	Chromosome designation	Centromeric index
Karadi	I	Metacentric	40.8
	I	=	44.7
	2	=	47.05
	2	=	42.4
	3	=	44.I
	3	=	46.6
Awassi	I	=	44.I
	I	=	50
	2	=	41.6
	2	=	43.4
	3	=	46.I
	3	Submetacentric	28.I
Arabi	I	Metacentric	43.5
	I	=	44.2
	2	=	46.3
	2	=	43.2
	3	=	47.2
	3	=	42.7

72 or 48 hours and that PHA prepared two dyas before use and stored at 4 °C was more active than freshly prepared material.

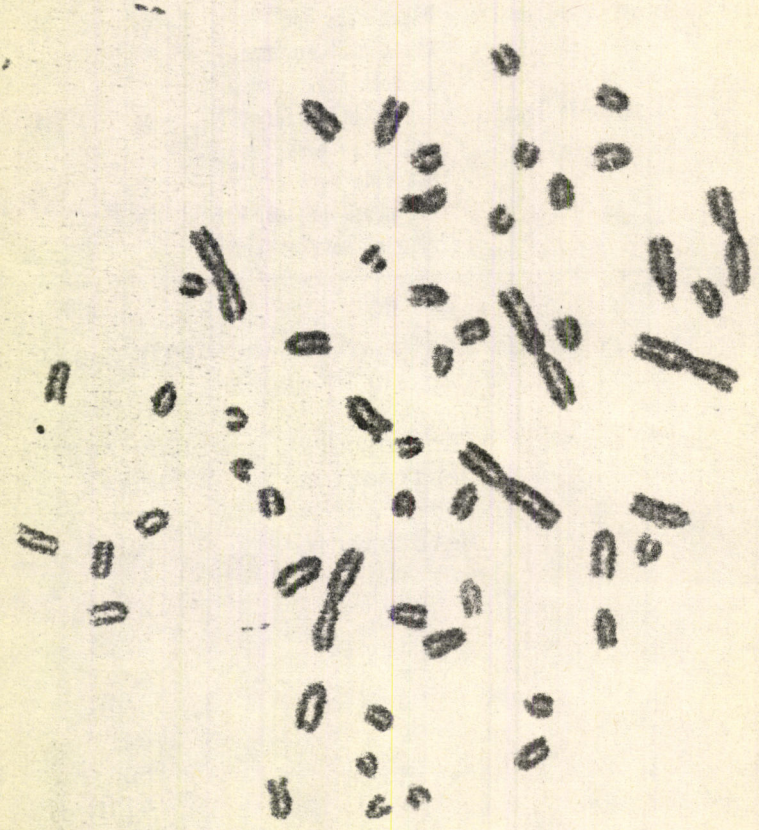

Figure 1: Chromosome spread of Karadi breed.

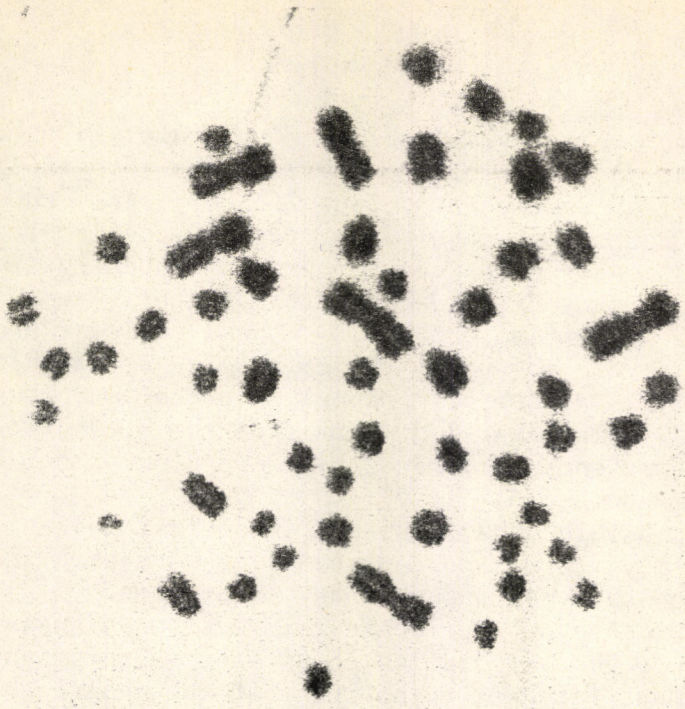

Figure 2: chromosome spread of Arabi breed.

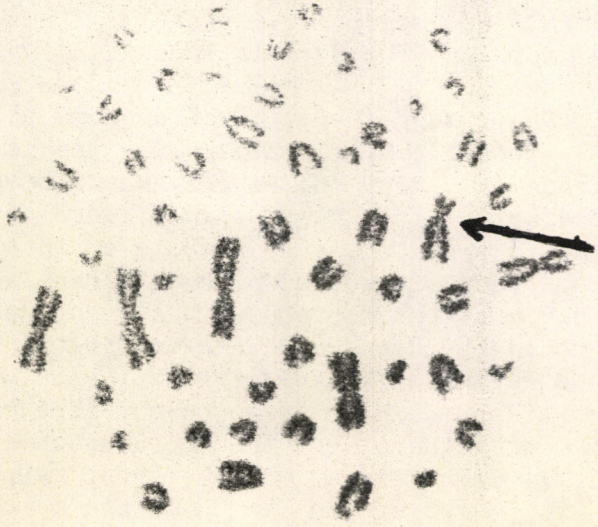

Figure 3: Chromosome spread of Awassi breed (Note one submetacentric chromosome arrowed.

DISCUSSION

It is well established that the diploid chromosome number of domestic sheep (Ovis aries) is (54XX,54XY) consisting of 3 metacentric and 24 telocentric chromosome (6,7). Counts of chromosome for 50 cells from each individual subjected to the present investigation have shown the same diploid chromosome number (Figs. 1,2,3). with the exception of Awassi breed, Karadi and Arabi breeds have shown a typical Ovis aries karyotype (Figs. 1,2) in term of chromosomal number and morphology (Table 2). The Awassi differs from the above two breeds by the presence of pericentric inversion on one copy of chromosome number 3 (Fig.3).

It is possible that pericentric inversion like centric fusions may form spontaneously in domestic sheep and cattle and this may occur at any chromosome.

The occurrence of pericentric inversion in Awassi breed has led to the speculation about their significance . It is of great interest to notice that the Awassi which has shown a heterozygous polymorphism on one copy of chromosome number 3 lives normally in the middle of the country. The Karadi and the Arabi breeds are concentrated in the north and south respectively (Table 1). Such observation may provide correlations with environmental variable .

Other worker have suggested that chromosome polymorphism may have a possible implication in the selection of certain breed of sheep (8) as the Awassi breed in this case. Accordingly, the real significance of inversion polymorphism is unknown since we have no information as to the actual routes through which different chromosomal states actually affect fittness. More cytogenetic study, however, should be conducted to confirm if there is a clear cut association between the pericentric inversion and the Awassi breed which lives mainly in the middle of the country or wether there are other group of the same breed which might show normal sheep karyotype or homozygous polymorphism on the same chromosome and the significance of such finding if any.

Table 3: Development of sheep leucocyte culture technique

Culture media	Phytohaemaglutinin	Comment
TC 199XI	0.5	No metaphase spreads
	0.I	= = =
	0.I	Very few
	0.2	= =
BME XI	0.5	No metaphase spreads
	0.I	Very few
	0.I5	few metaphase spreads
	0.2	many = =
MEM XI	0.5	No metaphase spreads
	0.I	= = =
	0.I5	= = =
	0.2	= = =

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دلالة تعدد الاشكال الكروموسومية في سلالة واحدة من الاغنام العراقية

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الخلاصة

اجري التحليل الكروموسومي لثلاثة سلاسلات من الاغنام العراقية (الكرادي، العرابي، والعواسي) باستخدام زرع الخلايا اللمفاوية في انابيب الاختبار حيث وجد ان العدد الكامل للسلالات الثلاث هو (2n=54xy/54xx) لكل من الذكور والاناث كما لوحظ ان نسخة واحدة من كروموسوم (٣) الاغنام العواسي يختلف عن بقية كروموسومات الاغنام العراقية الاخرى المستأنسه بحركة السنترومير نحو الاعلى. ان الوسط الغذائي المتكون من (Basal minimal medium) مضافا اليه مصل جنين الابقار هو الاكثر ملاءمه لزيادة انقسام الخلايا اللمفاوية في الاغنام لاغراض التحليل الكروموسومي.