Predicting some traits of the reproductive performance for Al-Awassi ewes through studying sex chromatin shapes in the blood

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ABSTRACT

The research was conducted in the field of the Department of Animal Production, College of Agriculture, Al-Qasim Al-Green University in order to study the sex chromatin in white blood cells (neutrophils) and its relationship to the reproductive performance for a sample consist of 16 ewes. Blood samples were drawn, prepared, and examined to determine the presence, shapes, and measurements of the sex chromatin and its relationship to reproductive traits. The results showed that the percentage of existing sex chromatin in Al-Awassi ewes varied significantly ($P < 0.01$) which amounted to (21.25, 30.75, 16.00 and 32.00%) for the drum stick, Sessile nodule, Teardrop, and Small club, respectively. As for the horizontal and vertical axes for the nucleus and chromatin, it was found that there was a significant effect at the significant level ($P < 0.05$) of the sex chromatin shapes on the vertical axis and the nucleus area. While the results showed that there was no effect for the shape of chromatin on the number of lobes for the nucleus and the horizontal axis for the nucleus. As for the traits of chromatin, the study showed a significant effect at the significant level ($P < 0.01$) for the shapes of sex chromatin, its axes, and its area. The study also found that there is a significant variation ($P < 0.05$) in the fertility rate according to the shape of the sex chromatin, where the ewes whose chromatin with Teardrop shape in their blood achieved the highest fertility rate amounted to (2.77 ± 0.08 born/abdomen) followed by the chromatin with Small club shape (2.71 ± 0.06 born/abdomen). As for females who were distinguished by sex chromate of the type of Sessile nodule or drum stick, it achieved fertility of (2.61 ± 0.05 and 2.52 ± 0.06 born/abdomen), respectively.

Keywords: sex chromatin, egg white blood cell nuclei, fertility.

التنبؤ ببعض صفات الاداء التناسلي للنعاج العواسية من خلال دراسة اشكال الكروماتين الجنسي في الدم

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قسم تقنيات التحليلات المرضية, كلية الحلة الجامعة الاهلية، محافظة بابل، العراق.

الخلاصة

أُجرِي البحث في حقل قسم الانتاج الحيواني في كلية الزراعة/ جامعة القاسم الخضراء لدراسة الصبغين الجنسي في خلايا الدم البيض (العدلات) وعلاقتها بالاداء التناسلي لعينة مكونة من 16 نعجة. سُحبت عينات الدم وحُضرت وفُحصت لتحديد وجود الصبغين الجنسي وأشكاله وقياساته وعلاقته بالصفات التناسلية. بينت النتائج أن النسبة المئوية لتواجد الكروماتين الجنسي في النعاج العواسية تباينت معنوياً ($P<0.01$) وبلغت 21.25 و 30.75 و 16.00 و 32.00 % لكل من عصا الطبال وبروز بدون ساق ودمعة العين والشكل الهراوي بالتتابع. اما فيما يخص البعد الأفقي والعمودي للنواة وللكروماتين فوجد ان هناك تأثير معنوي عند مستوى معنوية ($P<0.05$) لاشكال الكروماتين الجنسي على البعد العمودي ومساحة النواة, بينما اظهرت النتائج عدم وجود تاثير لاشكال الكروماتين على عدد فصوص النواة والبعد الافقي للنواة. فيما يخص صفات الكروماتين فقد اظهرت الدراسة وجود تاثير معنوي عند مستوى معنوية ($P<0.01$) لاشكال الكروماتين الجنسي وابعاده ومساحته. كما وجدت الدراسة أن هنالك تبايناً معنوياً ($P<0.05$) في معدل الخصب باختلاف شكل الكروماتين الجنسي، إذ حققت النعاج التي شاع في دمها الشكل دمعة العين أقصى معدل للخصب (2.77 ± 0.08 مولود/ بطن) تلتها مثيلاتها ذات الشكل الهراوي (2.71 ± 0.06 مولود/ بطن)، أما الاناث التي تميزت بكروماتين جنسي من نوع بروز بدون ساق أو عصا الطبال فقد حققت خصباً قدره 2.61 ± 0.05 و2.52 ± 0.06 مولود/ بطن بالتتابع.

الكلمات المفتاحية: الكروماتين الجنسي, انوية خلايا الدم البيض, الخصب.

1. INTRODUCTION

Animal breeders resort to pursuing programs that raise the animal's productive capacity through improving its genotypes, but the period required for this is often long in sheep and goats (1), While breeders can use other methods to reach the goal and with the maximum possible time, and among these methods is the use of early selection for some traits that can be adopted for indirect indicators for the selection of important economic traits, including shapes and measurements of sex chromatin (3, 2). Each (4, 5, 6) indicated that in a female that carrying two copies of the (x) chromosome, one of these two chromosomes turns into dense chromatin called a Barr body, which is found on white blood cells with multiple nuclei (neutrophils), where Its shape and axes can be used as an important genetic marker. The shape and presence of sex chromatin is a genetic condition in which the external environment has no role in it (7,8). The evolution of the technique of studying sex chromatin has led to the possibility of linking among the genetic predisposition for some productive and reproductive traits for an animal and the traits and shapes of sex chromatin. As previous studies indicated the link between the traits of sex chromatin, fertility and reproductive problems in sheep (9,10,11), in goats (12), in cows (13,14), in poultry (15), in camels (16), in buffalo (17), and humans (18). Sex chromatin is found in the white blood cells for sheep and goats in four shapes: the Drum Stick, Sessile Nodule, Tear Drop and the Small Club (19).

2. MATERIALS AND METHODS

2.1 Animals of experiment and herd management

The research was conducted on Al-Awassi ewes as well as utilization of the breeder's data to study the traits of sex chromatin and its relationship to performance. Sheep are raised in semi-open barns dedicated to harboring them. Green and dry feeds are provided. The animals are subject to a preventive health program that includes immersing the animals using a pyrethroid cypermethrin solution (produced by Al-Masani Company for manufacturing Veterinary, Jordan) at a concentration of 10% to eliminate external parasites.

2.2 Collection of blood samples

Blood was collected from the jugular vein with amount 10 ml for each sample, and the blood was taken before the animals went out to graze using sterile needles attached to the plastic holder inside the evacuated tubes containing the anticoagulant (Ethyl Diaminic Tetra Acetic, EDTA). To prevent the occurrence of blood clotting, the tube rotates immediately after collecting the blood for one minute to mix the blood with the anticoagulant, and then the animal number is fixed on the tube and then transferred to the laboratory of animal physiology.

2.3 Preparing blood swabs

A small drop of blood was taken by using a clean fine pipette, the drop was placed on one end of a clean glass slide that washed with hot water and soapy and then dried with a small piece of cloth. The blood drop was then spread on the glass slide using another glass slide, Its edge was placed in front of the blood drop orthogonally and at an angle of 45 degrees, then pushed the slide towards the blood drop until it touched it to spread the blood drop along the edge of the slide by the capillary property, then the blood drop spread on the surface of the first glass slide consistently and at one speed. The blood swab was quickly dried with air by shaking it multiple times with the forefinger and thumb.

2.4 Staining the blood swabs (20)

The glass slides were placed on a metal stand and fixed with methanol alcohol before adding the necessary stain to it, which is the Wright-Geimsa stain, which was then lightly diluted by mixing 5 ml of stain with an equal amount of buffer solution in the test tube. The drops of the prepared solution were then placed on the glass slides in a manner that completely covered the

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blood swab. The blood swabs were then left to complete the staining and for ten minutes, they were then washed with distilled water and dried in the air, the fixing was then done by placing several drops of Canada balsam on the slide, The glass slides are kept in their own sealed plastic boxes to prevent the dust from reaching it until the examination is conducted.

2.5 Examining blood swabs and chromatin measurements:

The prepared blood swabs using the optical lens (X100) were examined using an ocular lens with a magnifying force of X20. The Meander System was used in the examination. where 100 neutrophil cells were calculated to determine the percentage of sex chromatin found in these cells and the percentages of its various shapes represented by the drum stick, Sessile nodule, Teardrop, and Small club. The Vertical and Horizontal Axes and Chromatin areas were also measured by replacing one of the optical lenses in the microscope and fixing an ocular micrometer in place of it. The unit used to measure the vertical and horizontal axes is a micrometer, as well as calculating the number of lobes for each nucleus in which the sex chromatin appeared as shown in Figure (1, 2), the method recommended by (4) was used, which included calculating the area of sex chromatin according to the following equation:

The area of sex chromatin (μm^2)= (a x b x π),

where π: is the fixed ratio 3.14, and a and b represent half of the vertical and horizontal axes, respectively.

2.6 Imaging

Microscopic images of various forms of sexual chromatin were taken using a specially designed camera attached to one of the optical lenses for the optical microscope (Sawyer microscope company, China).

2.7 Statistical analysis

Statistical Analysis System (SAS, 21) was used in data analysis to study the effect of different sex chromatin shapes on the axes of the nucleus and stains measured according to a complete random design (CRD), and the significant differences between the averages were compared using Duncan,s New multiple range test, The chi-squared test was also used to compare the significant differences between the percentages for the distribution of chromatin shapes in the sample. The coefficient of correlation was also extracted between the different traits.

Mathematical model:

$Yij = \mu + Fi + eij$

where

Yij: Observation value j for shape i.

μ: Overall mean for the studied trait.

Fi: effect of chromatin-shape i.

eij: the experimental error that distribution normally with a mean equal to zero and a variance of σ^2e.

3. RESULTS AND DISCUSSION

3.1 Preparing sex chromatin and distribution percentage of its shapes

Table (1) shows the numbers of sex chromatin and the distribution percentage of its shapes in the studied Al-Awassi ewes sample. It was found that the percentage of existing sex chromatin in Al-Awassi ewes varied significantly (P <0.01) which amounted to (21.25, 30.75, 16.00 and 32.00%) for each drum stick as shown in Figure (3), Sessile nodule as shown in Figure (4), Teardrops shown in Figure (5), and Small club as shown in Figure (6), respectively. These percentages show the presence of the Small club shape with a higher percentage than the rest of the sex chromatin shapes in the studied ewe samples, as well as what it indicated by (23), which stated that the percentage of existing sex chromatin may increase with progressing the age of the

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cells. The results of the current study did not agree with the results of previous studies that were conducted on different agricultural animals, where a high percentage of the Sessile nodule shape was recorded in the study (10) on sheep 3.55%, as well as in study (13) on Friesian and Holstein cows (2.4 and 2.65%), respectively. While (24) in their study on sheep found that the percentages of the drum stick, Sessile nodule, Teardrops, and Small club were (24.37, 64.49, 9.25 and 1.89%), respectively. As for the lowest percentages of sex chromatin shapes, this study agreed with (4) which found that the percentage of the Teardrops shape was (0.7%). As for the majority of the previous studies, it was shown that the appearance of the Small club shape in white blood cells (neutrophils) in sheep was low which amounted to (1.58%) (10) and (1.89%) (24), While (25) did not find the Small club shape in the nuclei of white blood cells (neutrophils) for sheep. The reason for the variation in the percentages of sexual chromatin shape from one study to another may be attributed to the difference in the type of animal and strain and the state of the herd as well as the size of the studied sample.

Table 1: Numbers of sex chromatin and the distribution percentage of its shapes in white blood cells (neutrophils) for Al-Awassi ewes.

The shape of sex chromatin	**Number**	**Percentage (%)**
Small club	128	32.00
Sessile nodule	123	30.75
Drum stick	85	21.25
Teardrops	64	16.00
Total	**400**	**%100**
Value of Chi-squared (χ^2)	---	** 8.067
****(P<0.01)**		

3.2 Relationship of Sex-Chromatin shapes with the number of lobes and the axes of the nucleus

Table (2) related to the relationship of Sex-Chromatin shapes with the number of lobes and the axes of the nucleus in Al-Awassi ewes shows that there is a significant effect at the significant level of (P <0.05) for the shape of sex chromatin on the vertical axis and the area of the nucleus, While the results showed that there was no effect for the shape of chromatin on the number of lobes for the nucleus and the horizontal axis of the nucleus. The arithmetic mean within the ranges reached in sheep (10,18) and the results of the current study are close to (26), where the overall mean for the horizontal and vertical axes and the area of nucleus amounted to (11.56 μm, 11.33 μm, 103.12 μm^2), respectively, While the mean of the horizontal and vertical axes for chromatin and the area of chromatin amounted to (1.30 μm, 1.02 μm, 1.08 μm^2), respectively, and It also approached the results indicated by (14) in the local Awassi shee

Table 2: Relationship of Sex-Chromatin shapes with the number of lobes and the axes of the nucleus.

The shape of sex chromatin	Average mean ± standard error			
	Number of lobes	Vertical axes of the nucleus (μm)	Horizontal axes of the nucleus (μm)	The area of the nucleus (μm^2)
Small club	0.09 ± 3.47a	0.13 ± 8.30a	0.12 ± 7.46a	28.80 ±0.16 b
Sessile nodule	0.07 ± 3.47a	0.12 ± 8.12ab	0.89 ± 8.51a	69.10±0.19 ab
Drum stick	0.09 ± 3.27a	0.19 ± 8.13ab	0.15 ± 7.40a	60.16± ab 0.12
Teardrops	0.09 ± 3.46a	0.18 ± 7.72b	0.15 ± 7.16a	55.27±0.16 a
Significant level	**NS**	*	**NS**	*
The means that have different letters within one column differ significantly among themselves. * (P <0.05), NS: not significant.				

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3.3 Relationship of the sex-chromatin shape with the axes and area of chromatin

As for the traits of chromatin, the study showed a significant effect at the significant level of (P <0.01) for the shapes of sex chromatin and its axes, and it is not observed any effect at the significant level of (P <0.01) for the shapes on the area of chromatin. As for (9), it was found that the means of the horizontal and vertical axes and the area of chromatin amounted to (1.30 μm, 1.02 μm, and 1.041 μm^2), respectively. The results of our study are agreed with (27) who mentioned that the area of chromatin is almost constant in most blocks, and if there are slight differences, it is caused by the differences in the size of the X chromosome (4).

Table 3: Relationship of the sex-chromatin shape with the axes and area of chromatin.

The shape of sex chromatin	Average mean ± standard error			
	Number	Vertical axes of the nucleus (μm)	Horizontal axes of the nucleus (μm)	The area of the nucleus (μm^2)
Small club	128	0.05 ± 1.19a	0.02 ± 0.681b	0.81 ±0.4 a
Sessile nodule	123	0.02 ± 0.993b	0.02 ± 0.859a	0.85 ±0.2 a
Drum stick	85	0.03 ± 1.151a	0.02 ± 0.710b	0.81 ±0.3 a
Teardrops	64	0.03 ± 0.741c	0.02 ± 0.606c	0.45 ±0.2 a
Significant level	---	**	**	NS
The means that have different letters within one column differ significantly among themselves. * (P <0.05), NS: not significant.				

3.4 The effect of sex chromatin shapes on the number of born resulting (fertility at born)

Table (4) shows that there is a significant difference (P <0.05) on the fertility rate according to the shape of the sex chromatin, where the ewes whose chromatin with Teardrop shape in their blood achieved the highest fertility rate amounted to (2.77 ± 0.08 born/abdomen) followed by the chromatin with Small club shape (2.71 ± 0.06 born/abdomen). As for the females, who were characterized by two sex chromatin of the Sessile nodule or drum stick, which achieved fertility of (2.61 ± 0.05 and 2.52 ± 0.06 born/abdomen), respectively. This result disagrees with (26) that showed fertility rate at its highest levels in ewes with sex chromatin of the Sessile nodule (1.46 ± 0.11 born/abdomen). It also disagreed with (24) that the highest fertility rate (1.43 born/abdomen) in the ewes with sexual chromatin of the Sessile nodule type, while the ewes carrying sex chromatin of the drum stick type were the lowest at the fertility rate. Fertility is the most important economic traits, and improving it through direct selection is slow and ineffective because it has a very low genetic equivalent, so resorting to indirect selection is one of the most important means to improve it. From the results of this study, measurements of sex chromatin can be adopted to improve the fertility rate in sheep.

Table 4: The effect of sex chromatin shapes on the number of born resulting (fertility at born).

The shape of sex chromatin	**Average mean ± standard error**
	Fertility rate
Drum stick	B 0.06 ± 2.52
Sessile nodule	B 0.05 ±2.61
Teardrops	A 0.08 ±2.77
Small club	A 0.06 ±2.71
Significant level	*
The means that have different letters within one column differ significantly among themselves.* (P <0.05),	

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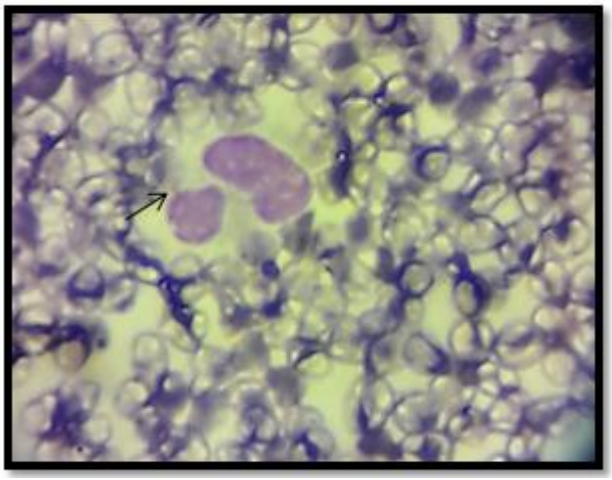

Figure 3: Sex chromatin of Sessile nodule type.

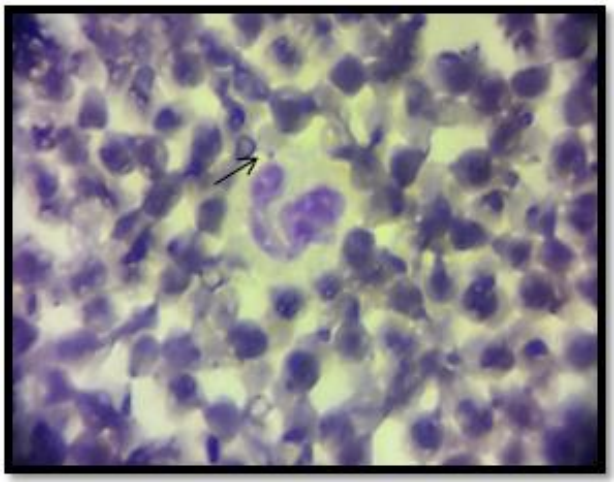

Figure 4: Sex chromatin of Drum stick type.

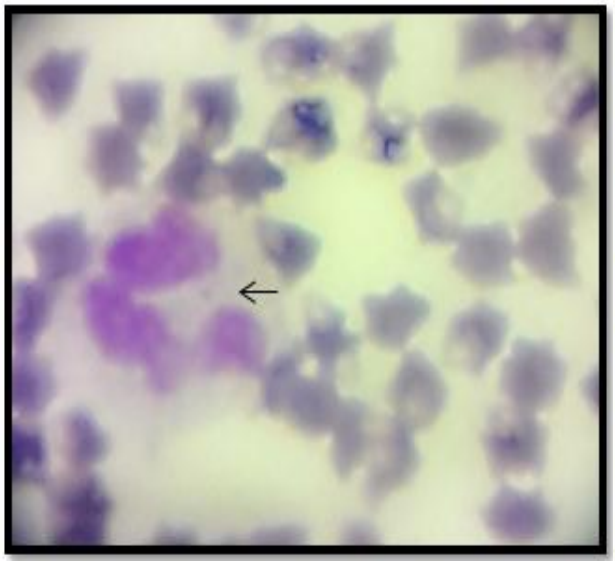

Figure 5: Sex chromatin of Small club type.

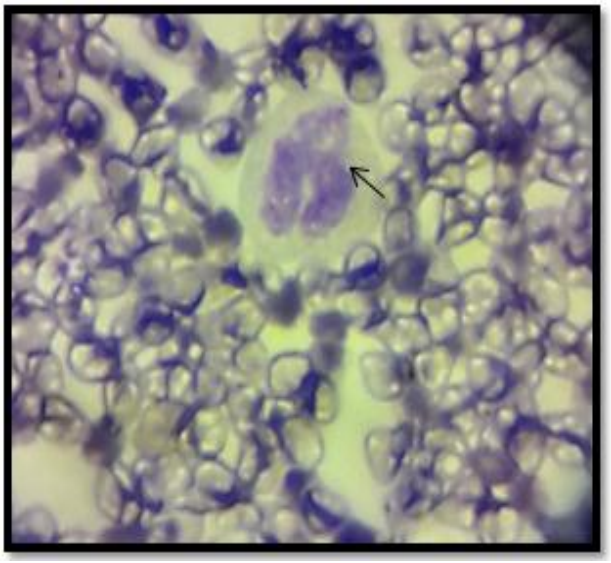

Figure 6: Sex chromatin of Teardrops type.

4. CONCLUSIONS

1- The possibility of improving the economic and reproductive traits (fertility) through the traits of the sex chromatin measured as an indirect method for selection.

2- It is possible to set selection plans and build genetic improvement strategies to accelerate improvement programs to develop herd performance and maximize economic returns.

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