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CYTOGENETIC STUDY OF THE HAMDANI SHEEP BREED OF THE IRAQI KURDISTAN REGION

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Abstract

This study presents a cytogenetic analysis of the local Hamdani sheep breed found in the Iraqi Kurdistan region, as it has an economic and agricultural industrial role. The main objective of this research was to understand the cytogenetic characterization involving the karyotyping of chromosomes (numbers and structure) of ten rams and fifteen ewes Hamdani sheep). The results indicated that the chromosomal numbers of 54(2n) and 52 chromosomes are autosomes with two sex chromosomes (XY in males and XX in females). The first three pairs of autosomes were submetacentric in both sexes. The relative lengths in the first three pairs in male sheep were 7 17,7,13 and 7.13µmcompared to 7,10,7.08 and 7.05µm for the females. The sheep's 4th to 26th pairs of chromosomes were acrocentric, and their relative lengths decreased progressively from the 4th to the 26th. For male sheep, the X chromosome was acrocentric with a relative length of4.48µm, whereas the Y chromosome was submetacentric at 3.30µm. Both X chromosomes in female sheep were acrocentric and had the same relative length of 3.46µm. The first three pairs of male chromosomes had the same arm's length ratio (ARM ratio) of1.00µm, and their centromere index was the same at 49.87µm. All first three pairs of female chromosomes had the same ARM ratio at 1.00µm,

and their centromere indexes were 49.98, 49.89, and 49.91µm, respectively. The karyological analysis of this sheep indicated normal chromosomal complements without any abnormalities. This normal chromosome profile indicates sheep with promising reproductive health and fertility status.

Keywords: Hamdani sheep, Karyotype, Ideogram, Relative length, Centromere index.

دراسة الوراثة الخلوية لسلالة الاغنام الحمدانية في أقليم كوردستان العراق

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الخلاصة

هذه الدراسة تبين التحليل الوراثي الخلوي لسلالة الأغنام الحمداني المحلية في إقليم كردستان العراق لما لها من دور اقتصادي وصناعي، وكان الهدف الرئيسي من هذا البحث هو فهم التوصيف الوراثي الخلوي، اي التنميط الكروموسومي (العدد والهياكل)، فقد تم اخذ 25 عينة الدم من (عشرة اكباش وخمسة عشرة نعجة والنتائج تبين أن عدد الكروموسومات هي (2n=54)، حيث تتضمن 52 كروموسوما جسميا مع كروموسومين جنسين XY في الاكباشو كروموسومين جنسين XX في النعاج، وان فقط الأزواج الثلاثة الأولى من الكروموسومات الجسمية تمتلك سنترومير ذات موقع شبه وسطي في الاكباش والنعاج، ومعدل اطوال النسبية لهذه الازواج في الاكباش هي (7.17، 7.13، 7.13) µm وعلى التوالي، اما في النعاج فكانت 7.10، 7.05، 7.08 µm وعلى التوالي، والكروموسومات المتبقية في الاكباش والنعاج هي كروموسومات طرفية السنترومير ومعدل اطوالهن النسبية تتناقص تدريجيا. اوضحت النتائج ان كروموسوم X في الاكباش كانت طرفية السنترومير وبطول نسبي 4.48 µm، وكان كروموسوم Y ذات سنترومير شبه وسطي بمعدل طول نسبي 3.30 µm، وفي النعاج كلتا الكروموسومين X كانتا طرفية السنترومير وبطول نسبي 3.46 µm، اظهرت البيانات ان النسبة المئوية لطول ذراع الكروموسومات الثلاث الاول للأكباش كانت 1.00 µm وان سنترومراندكس كانت 49.87 µm، اما في النعاج فان النسبة المئوية لطول الذراع هذه كروموسومات لها قيم متشابهة والتي هي 1.00 µm ومعدل سنترومراندكس لهذه الكروموسومات الثلاث كانت 49.98، 49.91، 49.89 µm وعلى التوالي. أظهر التحليل الكاريولوجي ان جميع الكروموسومات هي طبيعية دون أية تشوهات وأن الأغنام تتمتع بصحة إنجابية وبحالة خصوبة جيدة.

كلمات مفتاحية: الاغنام الحمدانية، التركيب الكروموسومي، الايدوغرام، الطول نسبي، دليل سنترومير.

Introduction

Sheep are the primary livestock in Iraq and play a crucial role in the country's economy. They are raised for various purposes, such as meat, milk, and wool, with each product contributing significantly to overall annual incomes at approximately 60%, 25%, and 15%, respectively (15). Hamdani sheep, described as the largest Iraqi sheep breed with a high twinning rate and large body frame, have a potential milk yield of 83.998 kg per lactation (1), a growth rate of 148 gm per day (3), and coarse wool production of 2.159 kg per year (2). It has a broad range of long wide pendulous ears and the absence of horns in both sexes (22). Product quality, as determined by genetic potential, is a key factor in determining the economic efficiency and profitability of the sheep breeding industry; however, the cytogenetic underestimation of sheep is a crucial agricultural object (11). Ravichandran (22) reported that the organism's complete set of chromosomes is called the karyotype. It is usually presented as a demonstration of the metaphase chromosomes arranged in descending order of size. Chromosome identification in sheep is also important because of its genetic significance for breeding and subsequent breeding programs. Compared to cattle and goats, sheep have fewer chromosomes and are smallerin size (11). Improving the genetic potential of animals is crucial for enhancing their productivity. The number and morphology of chromosomes are regarded as fundamental requirements for a thorough understanding of the genetics of a species (7 and 18). In domestic animals, chromosome number abnormalities are rarely reported, as they exhibit abnormal animal phenotypes and are quickly eradicated by breeders because they are frequently phenotypic and undetectable. Structurally balanced chromosome anomalies and numerical abnormalities with sex chromosomes have been detected more frequently (11). According to Mandal (18), cytogenetic analysis of domestic animals is useful in biotechnology to improve livestock genetics. It is also used to detect chromosome aberration that cause abnormal body conformation, congenital anomalies, decreased fertility (balanced chromosome abnormalities), or sterility (sex chromosome abnormalities). To identify the number and structural chromosome anomalies, a karyotyping analysis of goat and sheep breeds is highly important for determining their cytotogenic relationship (7 and 11). Although many studies have been carried out to characterize the phenological and molecular genetic characteristics of Hamdani sheep, very little is known about cytogenetic traits. The analysis of the karyotype of sheep is theoretically and practically important since the development of all the organism's features is determined by the genes in the chromosomes, and chromosomal abnormalities of any type may causea decrease in economic value and reproductive function (8). The main objective of this study was to conduct an initial cytogenetic assessment of the Hamdani sheep breed native to Iraq, to determine its conformity with the established karyotype standard for the species; additionally, the study evaluated the feasibility of initiating a cytogenetic screening program tailored for this specific breed.

Materials and Methods

This research involved karyotyping 25 Hamdani sheep (ten rams and fifteen ewes) from the Sebardani Ado-Qushtab a private animal farm in Erbil governorate of the Kurdistan region of Iraq. Chromosomal Analysis (9) of each animal was done using three ml of peripheral blood that was collected in vacuum tubes containing lithium heparin, an anticoagulant (VACUTEST KIMA S.r.l -Italy). Then 4.5 ml of a chromosomal medium (Gibco™ PB-MAX™ Karyotyping Medium) was mixed with 0.5 ml of each blood sample and incubated at 37°Cfor 71 hours. Next, 100 µl of colchicine (gibco, USA) was added for about 1 hour before the end of the culture and centrifuged. The recovered pellet was treated with a hypotonic solution 0.075 M KCl and incubated at 37°C for 10 minutes. Samples were centrifuged and secured with an acetic acid/methanol fixation (1:3 v/v). This procedure was repeated three to four times: 2-3 drops of cell suspension were dropped from a height of three feet onto the clean tilted slide at a 45° angle, and then the edge of the slide was held on the bench. These dried slides were absorbed in 2.5 ml trypsin solution (Trypsin-EDTA 1X in PBS, Euroclone S.P.A. Italy) with 50 ml of normal saline for one minute at 37°C. Subsequently, they were stained with Giemsa when dried fluorescent dyes that use quinacrine were applied to determine the specific chromosomes and their structure anomalies based on a banding pattern. Each chromosome can be identified accurately using the characteristic banding pattern (12).

An individual chromosome's relative length (RL) is calculated as the ratio of its length to the total length of the haploid set of chromosomes (10 and 24).

Relative length = (Length of individual chromosome / total length of chromosome in the haploid set) ×100

The Centromeric Index was determined by taking the ratio of the short arm's length (p) to the total chromosome length (p+q) and multiplying the result by 100 (10 and 24).

Centromeric index (CI) = [Small Arm / (Long Arm+Small Arm)] ×100

The arm ratio is determined for chromosomes with arms using the formula outlined by (10 and 24).

Arm ratio = [Length of long arm (q) / Length of short arm (p)] × 100.

Results and Discussion

The results of this study reveal the presence of 54 (2n) chromosomes in Hamadani sheep, including 52 autosomes and two sex chromosomes XY and XX in females (Figures 1 and 2). These are confirmed by several previous studies conducted on different breeds of sheep (6, 17, 19 and 25). In addition, other studies using Karyological analysis with the help of cytogenetic technology reveal that the diploid set of chromosomes in various breeds of sheep includes 54 chromosomes, 26 pairs of autosomes, and 1 pair of sex chromosomes (XX or XY) (4, 14 and 24). Chromosome profiling can be a useful tool for identifying the reproductive health and fertility status of breeding animals early on (4 and 5). It has been confirmed that cytogenetic studies can assist in selecting animals without chromosome abnormalities such as poor body shape, low fertility, or infertility

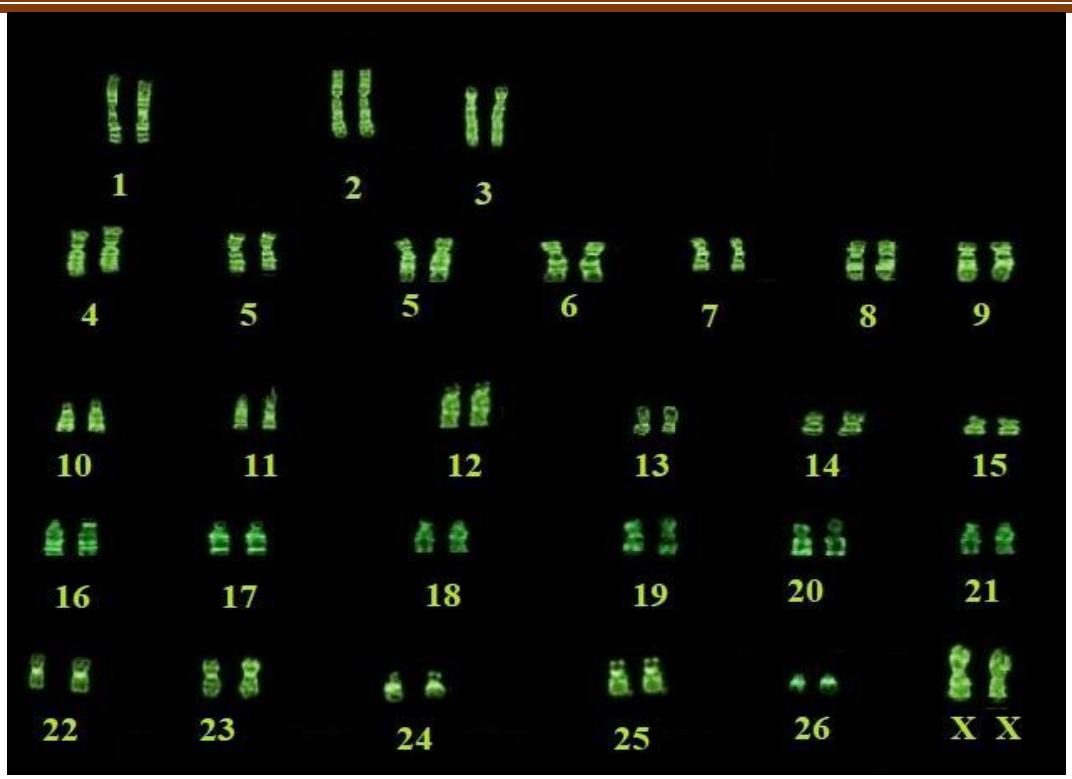

Fig. 1: Karyotype of chromosomes in female Hamdani sheep using quinacrine stain.

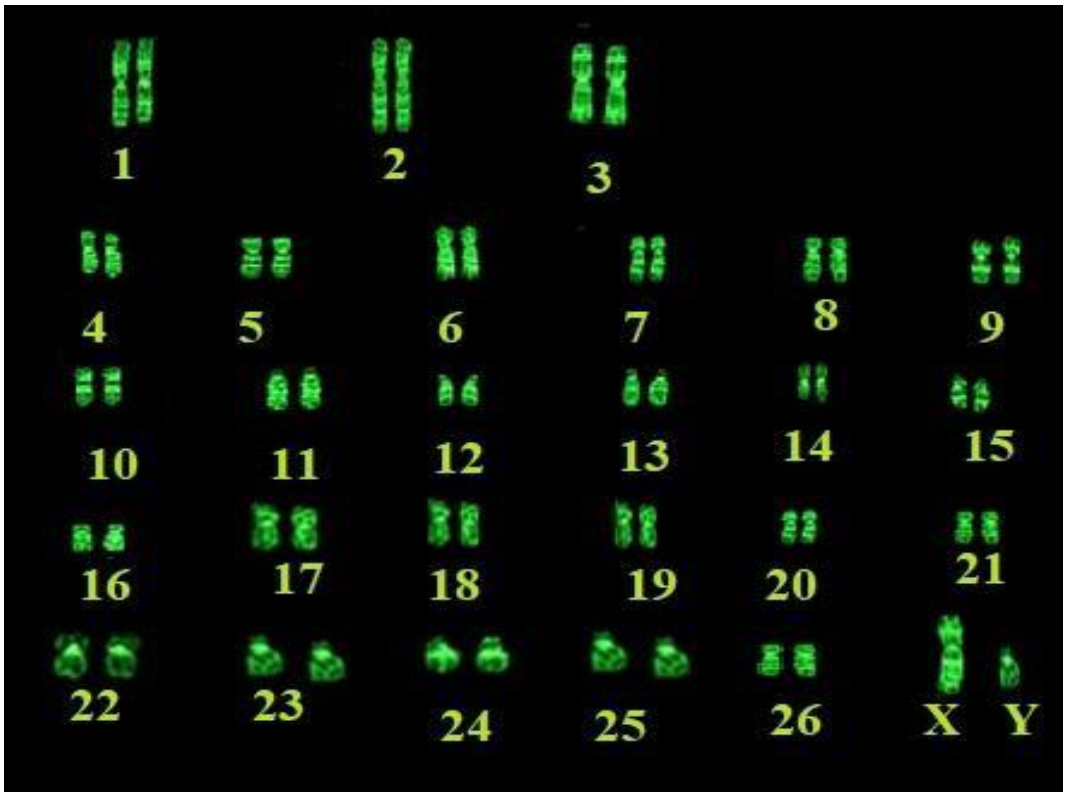

Fig. 2: Karyotype of chromosomes in male Hamdani sheep using quinacrine stain.

Based on the position of the centromere, it was observed that only the first three pairs of autosomes in both the male and female sheep were submetacentric. The male sheep had a mean relative length of 7.17 µm in the first pair, and 7.13 µm in the second and third pairs. The relative lengths of the first, second, and third pairs of chromosomes in the female sheep were 7.10, 7.08, and 7.05 µm, respectively (Tables 1 and 2). The other pairs in male sheep were all acrocentric and gradually dropped in relative length from the 4th (3.64µm) to the 26th pair (2.92 µm), while the relative lengths in the females declined gradually from 3.50 µmin the 4thpair to 2.86 µmin the 26th pair (Figure 3). Previous studies (20, 21, 23 and 25) indicated similar results claiming that the first three autosome pairs were the largest and submetacentric while the others were acrocentric. According to a study, a shift or reciprocal translocation in the chromosomal arms during the process of evolution may be responsible for the differences in the morphology of armed chromosomes found in different breeds of sheep (13). Research showed that the sheep of the Ukrainian Mountain-Carpathian breed have chromosomes that can be divided into two groups based on their morphological structure, i.e., acrocentric and metacentric, with a series of autosomes represented by three pairs of the large metacentric chromosomes (5). The remaining 23 pairs form a series of acrocentric chromosomes that gradually decrease most containing a centromere at the terminal end.

The X chromosome in Hamdani sheep was acrocentric with a relative average length of 4.48 µm while the Y chromosome was submetacentric with a relative average length of 3.30 µm in male Hamadani sheep (Table 1). Both X chromosomes in female sheep were acrocentric and had a relative length of 3.45 µm (Table 2). These results were also seen in earlier studies (13, 16 and 23). Finding the first pair is the largest submetacentric (5). The X chromosome for sex is acrocentric at the same time. The relative lengths of the X and Y chromosomes vary widely in the literature (13), indicating the Y chromosome is a submetacentric.

Table 1: Relative chromosome lengths of male Hamdani sheep.

Chromosome Pair Number	Relative Length (µm)	ChromosomeType
1	7.17	Submetacentric
2	7.13	Submetacentric
3	7.13	Submetacentric
4	3.64	Acrocentric
5	3.52	Acrocentric
6	3.50	Acrocentric
7	3.48	Acrocentric
8	3.48	Acrocentric
9	3.47	Acrocentric
10	3.45	Acrocentric
11	3.43	Acrocentric
12	3.41	Acrocentric
13	3.37	Acrocentric
14	3.36	Acrocentric
15	3.34	Acrocentric
16	3.30	Acrocentric
17	3.28	Acrocentric
18	3.26	Acrocentric
19	3.25	Acrocentric
20	3.23	Acrocentric
21	3.19	Acrocentric
22	3.15	Acrocentric
23	3.08	Acrocentric
24	3.01	Acrocentric
25	2.93	Acrocentric
26	2.92	Acrocentric
X	4.48	Acrocentric
Y	3.30	Submetacentric

Table 2: Relative chromosome lengths of female Hamdani sheep.

Chromosome Pair Number	Relative Length (µm)	Chromosome Type
1	7.10	Submetacentric
2	7.08	Submetacentric
3	7.05	Submetacentric
4	3.50	Acrocentric
5	3.50	Acrocentric
6	3.48	Acrocentric
7	3.47	Acrocentric
8	3.45	Acrocentric
9	3.44	Acrocentric
10	3.42	Acrocentric
11	3.40	Acrocentric
12	3.38	Acrocentric
13	3.35	Acrocentric
14	3.31	Acrocentric
15	3.29	Acrocentric
16	3.27	Acrocentric
17	3.27	Acrocentric
18	3.24	Acrocentric
19	3.22	Acrocentric
20	3.20	Acrocentric
21	3.18	Acrocentric
22	3.13	Acrocentric
23	3.06	Acrocentric
24	2.98	Acrocentric
25	2.91	Acrocentric
26	2.86	Acrocentric
X	3.46	Acrocentric
X	3.46	Acrocentric

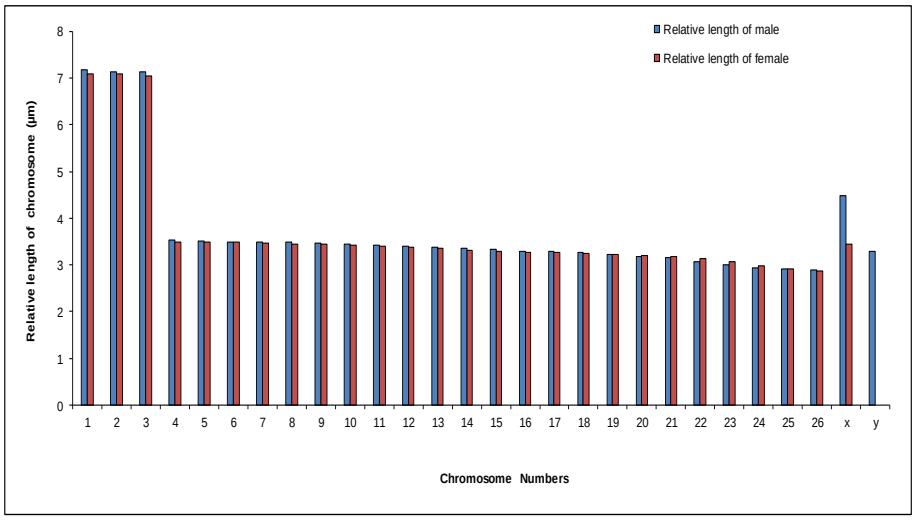

Fig. 3: Ideogram of Hamdani sheep.

Table 3 shows the ARM ratios for the first three pairs of autosome chromosomes in male Hamadani sheep. All three pairs had ratios of 1.01 µm, and their centromere index averages (%) were 49.87 µm, respectively. The ARM ratio average percentages (q/p) for the first three pairs of autosome chromosomes in the female sheep were 1µm, respectively while their centromere index averages were 49.98, 49.89 and 49.91 µm (Table 4). The ARM ratio's magnitude denotes the chromosomes' nature (25). Studies have demonstrated small differences between male and female sheep breeds in the ARM ratio and Centromere index (10, 13, 16 and 25).

Table 3: ARM ratio and Centromere Index of the first three chromosomes of male Hamdani sheep.

Chromosome Pair Number	ARM length ratio (%) (q/p) (µm)	Centromere Index (%) (µm)
1	1.01	49.87
2	1.01	49.87
3	1.01	49.87

Table 4: ARM ratio and Centromere Index of the first three chromosomes of female Hamdani sheep.

Chromosome Pair Number	Arm length ratio (%) (q/p) (µm)	Centromere index (%) (µm)
1	1.00	49.98
2	1.00	49.89
3	1.00	49.91

Conclusions

The cytogenetic evaluation in this study revealed that the diploid chromosome number of the Hamdani sheep breed was 2n = 54, consisting of 26 pairs of autosomes and a pair of sex chromosomes, with the chromosomes of the first group exhibiting a submetacentric structure and being larger than the others. The X chromosomes are the acrocentric members, whereas the Y chromosome appears very small and of a submetacentric morphology. The chromosome organization provided by karyotyping can contribute to understanding genetic evolution and breed characterization, ultimately supporting efforts for conserving these sheep breeds and making cytogenetic studies useful for practical breeding purposes in sheep farming.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

D. M. Khdr performed the Karyotype and ideogram analysis, D. M. Khdr and K. J. Karim contributed to the idea, design, and execution of the study and determined the parameters of these analysis. D. M. Khdr and K. J. Karim assisted in all animal procedures for the experiment. both authors contributed equally to the write-up of the final manuscript.

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No Data Availability Statement.

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The authors declare no conflict of interest.

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