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Sequences whole mitochondrion genome of local arabi sheep in Basrah

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Abstract

Ovis aries is a vital livestock species in Iraq. The Arabi breed plays a significant role in the Iraqi national agricultural production. Despite their importance, genetic studies on Arabi sheep have remained limited. This study aimed to characterize, for the first time, the complete mitochondrial genome of the Arabi sheep breed in Basrah, Iraq. This is essential for understanding the species' genetic diversity and for improving their productivity and health. The findings will help enhance food security, reduce veterinary costs, and support the Iraqi rural economy. To clarify its genetic diversity and evolutionary relationships, blood samples were collected from 165 Arabian sheep. PCR amplified the mtDNA, which was sequenced and analyzed using DNASTAR SeqMan Ultra, BLAST, and MEGA12 software for genome assembly, mutation identification, and phylogenetic analysis. The complete mtDNA genome was 16,619 base pairs long, aligning closely with the lengths reported in other *Ovis aries* breeds. Three single-nucleotide variants were identified and deposited in GenBank under accession numbers LC649167, LC649168, and LC649169, showing over 99% similarity to the Hamdani breed, suggesting a close maternal lineage and a possible genetic admixture. The phylogenetic analysis, performed using the Neighbor-Joining method in MEGA12 with 1,000 bootstrap replications, revealed genetic affinity among the Arabi, Hamdani, and Lezgin breeds, supporting a common geographical origin. The mtDNA's slow mutation rate, functional constraints, and human-mediated breeding practices likely contributed to the observed genetic similarity among breeds. The identified mutations in various mitochondrial genes may influence energy metabolism, growth, and adaptation. This study provides valuable molecular data for the conservation and genetic improvement programs of Iraqi sheep. It highlights the importance of mtDNA analysis in understanding breed diversity and evolution.

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Introduction

Sheep are among the most important domesticated animals in Iraq; they were bred thousands of years ago for their products, including meat, dairy, and wool (1,2). Arabi sheep are considered among the most important breeds of Iraqi sheep. They are widely distributed across various regions in Iraq, and they contribute significantly to the sheep products of Iraq, such as milk and its derivatives, meat, and wool too (3). Despite this importance, genetic studies on this breed remain somewhat limited (4), which could partly account for its relatively lower productivity compared with global breeds, as many global breeds have been subjected to deliberate selection linked to modern studies such as molecular genetics (5). Genetic studies are a powerful and vital tool for understanding and improving farm animals, contributing to increased productivity, improved product quality, food security, and the preservation of genetic diversity (6). Molecular genetic studies of livestock have revolutionized animal husbandry in recent years. Mitochondrial DNA is a unique tool that sheds light on the maternal lineages of these animals and their evolutionary trajectories (7). Mitochondria are essential cellular organelles responsible for energy production; they also play

key roles in apoptosis and overall cellular health. Uniquely, mitochondria possess their own genetic material, known as mitochondrial DNA (mtDNA) (8). Studying the mtDNA genome can help understand the evolutionary lineages of sheep and determine their genetic relationships with other species or breeds (9). This could lead to the identification of genetically distinct breeds in terms of production and disease resistance (10). These studies may also help to identify and conserve rare or endangered breeds (11) and estimate the level of genetic diversity within breeds. Perhaps studying the entire mtDNA genome could effectively distinguish between local and exotic breeds (12). Understanding mitochondrial mutations may help diagnose energy- or metabolic-related genetic diseases in sheep (13), in addition to their effective role in regulating apoptosis (14). It is important to note that the mtDNA genome encodes several genes involved in energy production and cellular signaling; therefore, variations in mtDNA in sheep may influence disease response, metabolism, and growth (15). Several studies have linked genetic patterns to birth weight, development, and their response to various environmental stressors (16). The study of the mtDNA genome in sheep offers essential insights into maternal lineages, domestication processes, and evolutionary trajectories. Furthermore, mtDNA variation is closely associated with functional traits, including metabolism, growth, and overall health. Comprehensive genome analysis also improves taxonomic resolution among breeds and plays a pivotal role in guiding breeding programs and conserving genetic resources (17). The mtDNA length is approximately 16,617 base pairs long, consisting of 37 genes representing the D-loop region and a portion of it for rRNA (two genes), 13 protein-coding genes, and 22 tRNA genes (18). Although the mtDNA genome is relatively conserved and lacks introns, there is significant nucleotide diversity among sheep breeds, which contributes to our understanding of sheep evolution and domestication (19). In sheep, there are multiple mtDNA haplogroups that are shared across many breeds. This indicates genetic dispersal between breeds, regardless of their breeding regions. Sheep in Europe and Asia generally share the same genetic patterns, suggesting complex domestication and selection. (20).

Although universal researchers have thoroughly examined mitochondrial genomes across various sheep breeds, their studies are limited to crossbred Iraqi sheep; thus, they remain insufficient and hinder conservation and genetic improvement initiatives. This study aims to characterize the mtDNA genome of Iraqi Arabi sheep molecularly.

Materials and methods

Ethical approve

The study has been approved by the Animal Ethics Committee of the Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq, dated 01/01/2024.

Blood samples

Blood samples were collected from 165 local Arabi sheep (90 females and 75 males) raised by local farmers in Safwan district, Basrah, southern Iraq (approx. 30.1131° N, 47.7194° E). The blood was drawn through the jugular vein.

DNA Extraction and primers

Genomic DNA was purified from the leukocyte fraction of whole blood samples using the DNeasy® Blood & Tissue Kit (Qiagen, Germany), strictly adhering to the manufacturer's guidelines. Leukocytes were selected as they constitute the predominant source of nuclear DNA in mammalian blood. The purified DNA was eluted with nuclease-free water, rehydrated overnight at 4 °C, and the purity and quantity of the product were then verified using a nanodrop. The purification process was carried out, and the purified product was subsequently stored at −20 °C until further analysis (21). The reference sequence MF004242 was selected as it represents the complete mitochondrial genome of Iraqi sheep from the Al-Hamdani breed, which is genetically and geographically the closest to the Al-Arabi breed. This choice was made to enhance the accuracy and reliability of the analysis. For comprehensive coverage, the genome was divided into six segments of comparable length, and primers were designed using the NCBI Primer-BLAST program (22) to specifically amplify mitochondrial genome regions while avoiding nuclear mitochondrial sequences (NUMTs) (Table 1).

PCR protocol

PCR amplification was performed as described by Tan *et al.* (23), with a total reaction volume of 20 µL containing seven µL nuclease-free water, (20 ng/µL) 1 µL DNA sample, 10 µL 2x master mix (Phusion High-Fidelity PCR Master Mix, Thermo Fisher Scientific, USA), one µL primer (10 pmol/µL), each one is forward and reverse. The amplification conditions were as follows: 98 °C (initial denaturation, 30 s), followed by 35 denaturation cycles at 98 °C (30 s), annealing at 59-60 °C (according to each primer) for 30 s, and then 72 °C (extension, 90 s). The final step of the extension was conducted at 72°C for 5 minutes. The purification process was performed, and the products were sent for sequencing. The mtDNA genome assembly began with assessing the quality of the raw paired-end reads, followed by trimming low-quality bases and adapter sequences. Reference-guided assembly was then performed using DNASTAR SeqMan Ultra version 17.1, employing the *Ovis aries* mitochondrial genome from GenBank (Accession No. MF004242) as a template. To validate the assembly and identify potential novel variants, a complementary de novo assembly was conducted with IDBA-UD version 1.1.3, a tool optimized for managing variable sequencing coverage. The

outputs from both strategies were merged to generate the final consensus sequence, and read-depth analysis was applied to confirm the completeness and accuracy of the assembled genome. The obtained sequences were aligned using the BLASTn tool on the NCBI website, with the *Ovis aries* mitochondrial genome (Accession No. MF004242) as a reference. Alignments were performed with default parameters, an e-value threshold of 1e-5, a minimum identity of 99%, and coverage above 98%, ensuring precise detection of sequence similarity and reliable identification of nucleotide variants (24). The multiple sequence alignment (MSA) of the obtained sequences was conducted in MEGA version 12 using the ClustalW algorithm. The sequences were compared with the reference mtDNA genome of sheep (*Ovis aries*) retrieved from GenBank (Accession No. MF004242), and variations were detected directly through the alignment process. To infer evolutionary relationships, a phylogenetic tree was generated using the Neighbor-Joining (NJ) method in MEGA 12, with 1,000 bootstrap replications and reference sequences from several sheep breeds. Genetic distances were estimated using the Kimura 2-parameter model, providing a reliable framework for evaluating the phylogenetic relationships among the examined samples (25). The circular map of the Arabian sheep mtDNA genome (16.619 bP) was created in Geneious Prime (V. 1.2). The genome sequence data generated in this study were used after the raw reads were aligned to the *Ovis aries* reference genome using the Map to Reference feature. A read coverage plot was then added to show the distribution of coverage depth across the genome. Restriction enzyme sites were identified using the software's built-in restriction enzyme analysis tool (26). This map was used to illustrate the genome structure and enzyme site distribution and to ensure the accuracy and comprehensiveness of the analysis.

Table 1: The primers of fragments of mtDNA (22)

Gene	Fragment	Direction	Primer	length (bp)	temperature (°C)
MF004242	Fragment 1	Forward	CCCAAAACCTCCCACTCTCC	2770	60
		Reverse	ATGCTACCTTTGCACGGTCA		
	Fragment 2	Forward	GCTCTCATTGGAGCCCTACG	2770	60
		Reverse	TGGTTGATGCTTCTGTGGCT		
	Fragment 3	Forward	ACACGGGCTTACTTCACGTC	2770	60
		Reverse	ACTTCTTGCGCGTCTATGGT		
	Fragment 4	Forward	GGAGCCACCCTTGCACTAAT	2770	60
		Reverse	TGAACCGTAAACCCCGTCTG		
	Fragment 5	Forward	AACCATACCCATCGCAGCAA	2770	60
		Reverse	TTTGGGTGAGGGCGCATATT		
	Fragment 6	Forward	GCCCCACTATCAACACCCAA	2769	59
		Reverse	GCTCGTGATCTAGTGGACGG		

Results

The quality of the whole mtDNA genome amplification was evaluated using agarose gel electrophoresis (Figure 1). The genome was divided into six approximately equal fragments, each producing a distinct and well-resolved band without additional or nonspecific fragments. These findings demonstrate the efficiency of the amplification process and confirm that the DNA samples were of sufficient quality for subsequent sequencing and downstream analyses.

The complete mtDNA genome of the Arabian sheep was 16,619 bpairs, comparable to several other *Ovis aries* sheep breeds. Sequence analysis revealed three single nucleotide variants (SNVs) identified by comparison with the *Ovis aries* reference genome sequences (GenBank accession number: MF004242), These variations were found to result from several silent and missense mutations, distributed across several mitochondrial genes, including NADH dehydrogenase subunit genes (ND), cytochrome b (CYTB), ATP synthase subunit 8 (ATP8), and cytochrome c oxidase (COX) genes.

All detected variants have been registered in the GenBank database under accession numbers: LC649167, LC649168, and LC649169. These variants are caused by several mutations distributed across different regions of the genome, some in protein-coding genes such as ND2, ND4, ND5, and COX1, others in tRNA and rRNA genes, and the control region. Their effects may range from silent mutations that do not alter the amino acid sequence to missense mutations that can affect the function of proteins involved in bioenergy production and cellular activity. The identified single-nucleotide variants (SNVs) of the Arabi sheep mtDNA genome exhibited high sequence identity with the Hamdani reference gene (MF004242), ranging from 99.92% to 99.93% (Table 2).

Figure 2 shows the circular map of the complete mitochondrial genome of Arabi sheep, with a total length of 16,619 bp. The map illustrates the arrangement of the 37 mitochondrial genes, including 13 protein-coding genes, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and the control region. This organization highlights the structural composition of the mitochondrial genome and

indicates the locations of genes involved in energy production and essential cellular functions.

A phylogenetic tree analysis (Figure 3) using the Neighbor-Joining method with 1,000 bootstrap replications showed an apparent genetic affinity between the Arabi sheep and the Hamdani and Luzjin breeds, as well as a relative divergence between eastern and western breeds.

Analysis of genetic distances derived from the complete mitochondrial genome sequences revealed a pronounced genetic similarity among the examined sheep breeds. Using the Kimura 2-parameter model in MEGA 12, pairwise distances were estimated to range from 0.03% to 0.58%. Notably, the Arabi sheep showed the highest genetic proximity to the Hamdani and Luzjin breeds, with sequence identity exceeding 99.9%.

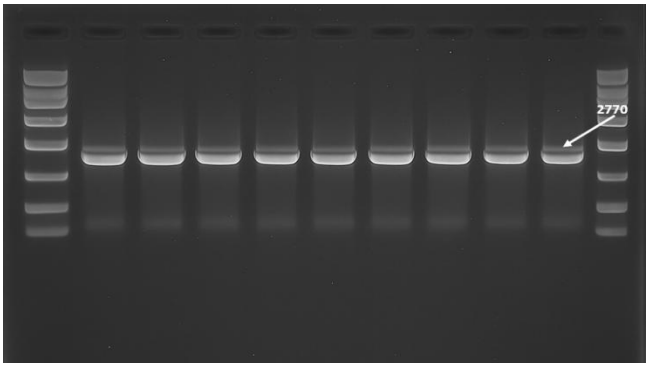

Figure 1: Agarose gel electrophoresis (1%) of the Arabi sheep mtDNA genome

Table 2: Sequence identity (%) between the reference gene (MF004242) and the identified single-nucleotide variants (SNVs) in the mitochondrial genome of Arabi sheep

No.	Accession No.	Sample size (n)	MF004242	LC649167	LC649168	LC649169
1	LC649169	78	99.92	99.93	99.93	100.00
2	LC649167	31	99.90	100.00	99.93	99.92
3	MF004242	Reference	100.00	99.90	99.92	99.92
4	LC649168	56	99.93	99.93	100.00	99.93

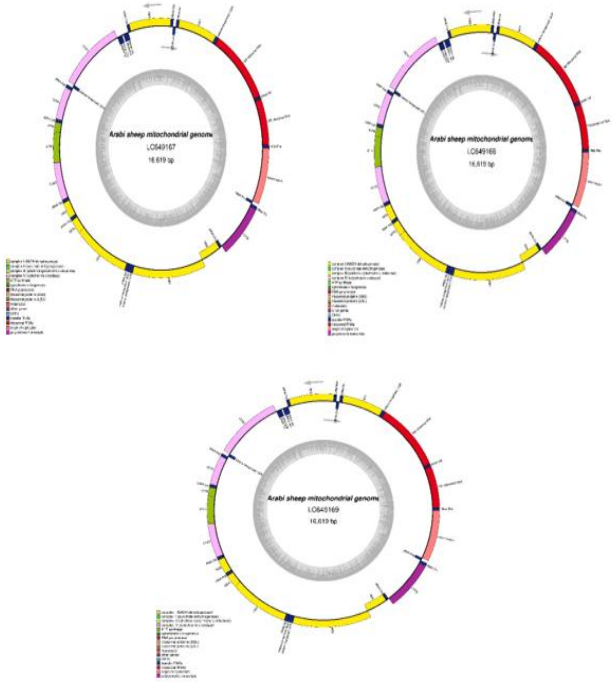

Figure 2: Circular maps of the complete mtDNA genome of Arabian sheep (16,619 base pairs), showing the organization of 37 genes, including 13 protein-coding genes, 22 tRNA genes, and two ribosomal RNA genes, in addition to the control region.

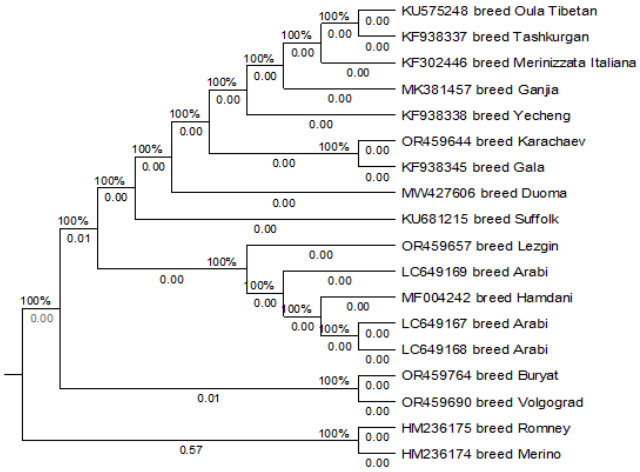

Figure 3: The phylogenetic tree of mtDNA genome sequences of different sheep breeds, including the Arabi breed, was constructed using the maximum likelihood method, showing a close genetic relationship between the Arabi, Hamdani, and Luzjin breeds.

Discussion

The similarity of Arabian sheep to other breeds (in neighbouring geographical areas) may be due to the closeness of their mtDNA genomes. (27). On the other hand, the results show that the Arabi and Hamdani sheep breeds belong to the same species (*Ovis aries*). However, this study

presents, for the first time, a comprehensive characterization of the mitochondrial genome of the Arabi sheep breed, identifying novel single-nucleotide variants (SNVs) and depositing them in GenBank. Although previous studies, such as Mustafa *et al.* (28), have indicated that most local sheep breeds, including the Hamdani and Karadi in Iraqi Kurdistan, share common maternal ancestry within known haplogroups, the present study provides detailed and accurate molecular data that can be used for genetic resource conservation and to strengthen future breeding and genetic improvement programs, The Hamdani and Arabi sheep breeds are maintained in geographically adjacent regions of Iraq, a factor that may have promoted genetic admixture and interbreeding between them. Consequently, the mitochondrial DNA similarities observed could be attributed not only to a shared geographic origin and common evolutionary background, but also to the impact of hybridization events. (29). It is also important to note that a slow rate of change characterizes the mtDNA genome in sheep, as it contains a small number of genes essential for survival, and it is relatively small; therefore, influential mutations are often harmful and do not persist, which leads to the genome being similar between breeds (30). The reason may also be human intervention in selecting and crossbreeding sheep for high productivity, which may reduce genetic diversity (31). The presence of multiple SNVs results from mutations occurring in distinct regions of the genome; this is common in the mtDNA and is considered an essential molecular marker in genetics and evolution (32). Contributing to the study of genetic diversity and evolutionary relationships among local breeds, mutations in different genes and locations within the sheep mtDNA genome may have a significant effect, depending on the functions of the genes in which they occur (33). The impact of missense mutations on the function of the resulting protein depends on the chemical nature and properties of the amino acids before and after the mutation (34). The occurrence of mutations in tRNA may affect translation accuracy, ribosomal binding, and the overall stability of the structure (35). Their presence in rRNA can influence both mRNA and tRNA binding sites, translation efficiency, and ribosomal unit composition (36). In general, mutations in rRNA and tRNA may indicate lineage differentiation, environmental adaptation, or thermal tolerance, and may affect growth rates or fertility (37). Moreover, mitochondria supply the energy required for sperm motility and membrane integrity, and similar associations have been reported in Awassi rams between seminal plasma enzymes, mineral concentrations, and semen quality (38).

On the other hand, mutations in the ND2, ND4, ND5, and ND6 genes are significant, as these genes encode subunits of NADH dehydrogenase, a critical enzyme complex in the oxidative phosphorylation pathway. This complex plays an essential role in mitochondrial energy metabolism, and variations within its subunits may therefore influence cellular energy production and adaptive responses to environmental stressors (39).

In this study, missense mutations were identified in the ND2 and ND4 genes. Since these genes encode essential subunits of NADH dehydrogenase, such amino acid changes may affect electron transfer efficiency and potentially alter the protein's three-dimensional conformation, consistent with previous reports (40). Although silent mutations were detected in the CYTB gene, their possible influence on mRNA stability, protein-binding regulatory sites, and translation efficiency indicates that these variants could contribute to functional diversity within the mitochondrial genome, aligning with the findings of this study (41). Silent mutations in the control region have also been reported; they may affect transcription and replication processes and, consequently, the mtDNA genome stability. Silent mutations have also been recorded in the control region, which may affect transcription and replication processes and secondary structures, thereby affecting mtDNA genome stability (31). In this study, a silent mutation was identified in the ATP8 gene. Although quiet, such a variant may potentially influence RNA secondary structure and translation efficiency (36). Moreover, mutations were identified in the COX1 and COX3 genes, both of which play essential roles in environmental adaptation. These alterations may, in some cases, impair mitochondrial energy production, thereby negatively affecting disease resistance and growth performance, as documented in earlier studies (24).

The diversity in the phylogenetic tree may be due to genetic mixing or hybridization. It can also be noted that there is an apparent genetic affinity between the Arabi breed and the Hamdani and Lezgin breeds, which may support the hypothesis that they share a common geographic origin. It can also be noted that there is an evolutionary divergence between the western and eastern breeds. Here, the importance of studying the mtDNA genome emerges in understanding the evolutionary relationships that play a vital role in maintaining genetic diversity and improvement of sheep (27,42).

This study provides the first complete mtDNA genome characterization of the Arabi sheep in Iraq, with novel variants deposited in GenBank, reveals low genetic diversity, and offers a valuable molecular reference for conserving genetic resources and advancing future breeding programs.

Conclusion

The presence of multiple single-nucleotide variants (SNVs) in Arabi sheep in Basrah Governorate, Iraq, may represent an essential indicator of genetic diversity within this breed and its evolutionary relationship with other breeds. This diversity may contribute to understanding responses to different environmental conditions, as it may serve as a molecular marker for selecting and genetic improvement programs. There is therefore a need for further studies

linking the mtDNA genome to different productions and physiological performances.

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Conflict of interest

The author discloses no potential conflicts of interest.

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تسلسل كامل الجينوم الميتوكوندري للأغنام العرابية المحلية في البصرة

حسن نعمة حبيب

قسم الإنتاج الحيواني، كلية الزراعة، جامعة البصرة، البصرة، العراق

الخلاصة

يُعدّ الأغنام العربية من الأنواع الحيوانية الحيوية في العراق. تلعب سلالة العرابي دورًا هامًا في الإنتاج الزراعي الوطني العراقي. وعلى الرغم من أهميتها، ظلت الدراسات الجينية على الأغنام العربية محدودة. هدفت هذه الدراسة إلى توصيف الجينوم الميتوكوندريا الكامل لسلالة الأغنام العربية في البصرة، العراق، لأول مرة. يُعدّ هذا ضروريًا لفهم التنوع الجيني للأنواع وتحسين إنتاجيتها وصحتها. ستساهم النتائج في تعزيز الأمن الغذائي، وخفض التكاليف البيطرية، ودعم الاقتصاد الريفي العراقي. ولتوضيح التنوع الجيني والعلاقات التطورية، جُمعت عينات دم من ١٦٥ رأسًا من الأغنام العربية. قام تفاعل البوليميراز المتسلسل بتضخيم الحمض النووي للميتوكوندريا، والذي تم تسلسله وتحليله باستخدام برامج DNASTAR SeqMan Ultra و BLAST و MEGA12لتجميع الجينوم وتحديد الطفرات والتحليل التطوري. بلغ طول جينوم الحمض النووي للميتوكوندريا الكامل ١٦٦١٩ زوجًا من القواعد، وهو ما يتوافق بشكل وثيق مع الأطوال المبلغ عنها في سلالات الأغنام العربية الأخرى. تم تحديد ثلاثة متغيرات أحادية النوكليوتيدات وإيداعها في بنك الجينات بأرقام الوصول LC649167 و LC649168 وLC649169، حيث أظهرت تشابهًا يزيد عن ٩٩% مع سلالة الحمداني؛ مما يشير إلى وجود سلالة أمومية وثيقة واحتمال وجود خليط وراثي. كشف التحليل التطوري، الذي أُجري باستخدام طريقة "الانضمام المجاور" في MEGA12 مع ١٠٠٠ تكرار تمهيدي، عن وجود تقارب وراثي بين سلالات العربي والحمداني واللزجين، مما يدعم أصلًا جغرافيًا مشتركًا. من المرجح أن معدل الطفرات البطيء في الحمض النووي للميتوكوندريا، والقيود الوظيفية، وممارسات التربية البشرية ساهمت في التشابه الجيني الملحوظ بين السلالات. قد تؤثر الطفرات المحددة في مختلف جينات الميتوكوندريا على استقلاب الطاقة والنمو والتكيف. توفر هذه الدراسة بيانات جزيئية قيّمة لبرامج الحفاظ على الأغنام العراقية وتحسينها وراثيًا.

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