Archives of Razi Institute, Vol. 78, No. 3 (2023) 941-947

DOI: 10.22092/ARI.2022.359888.2499

Original Article

Study of Local Black Iraqi Goats Genotypes for the *Cytb* Gene

Owaid, J. M[1 *], Yousief, M. Y[1], Abdulrda, A. J[1], Ayied, A. Y[1]

1. Animal Production Department, College of Agriculture, University of Basrah, Basrah, Iraq

Received 3 September 2022; Accepted 18 October 2022
Corresponding Author: affar.owaid@uobasrah.edu.iq

Abstract

Goats are the earliest domesticated ruminants. The local goat, *Capra hircus*, is considered one of the most important animals globally to provide good livestock production under harsh environmental conditions. This study aimed to detect the genetic structures of the local Iraqi goats bred in the central and southern regions of the country and investigate the possibility of benefiting from their genetic structures to construct improvement programs for increasing the productivity of these animals. To this end, blood samples were taken from 15 domestic black goats. A total of 10 ml of each animal's blood was placed in plastic containers of 10 ml. The DNA was extracted and sent to the laboratories of Juan Ju University, People's Republic of China, to analyze the sequences of the nitrogenous bases of the *Cytochrome b* (*Cytb*) gene. The results showed the presence of a genetic morphology for a segment of 670 base pairs for all the studied samples, and 15 sequences of this strain were recorded in the gene bank under the following accession numbers (LC496353.1:1-LC496367.1:1). The sequences of the nitrogenous bases of this segment of the gene, which were registered in the gene bank of some international goat breeds, were used for comparison with the sequences of black Iraqi goats to analyze the phylogenetic tree, calculate the genetic distance, study haplotypes, and calculate neutrality. The results showed the presence of one mutation in the studied segment of the *Cytb* gene, with a size of 670 bp. The mutation in base 46 of the studied gene converted from the purine group to the pyrimidine group (the shift from the nitrogen leaders A<C) in all the studied samples. It led to the transformation of the amino acid Asparagine into Histidine, where 233 amino acids were obtained, dominated by the amino acid Isoleucine and Leucine, over other amino acids at a rate of 14.34% and 11.21%, respectively. The phylogenetic tree showed the existence of two main branches, one of which included the local black Iraqi goat breed, and the other included all the international breeds under comparison. It is concluded that the black Iraqi goat breed has a different origin from other breeds.

Keywords: local black, Iraqi goats genotypes, *Cytb* gene

1. Introduction

Goats are the earliest domesticated ruminants. The local goat, *Capra hircus*, is considered one of the most important animals globally to provide good livestock production under harsh environmental conditions (1).

Goats are one of the animals that have not received enough attention in their breeding in most Arab countries and are still raised on the margins of agriculture. On the other hand, they have been efficiently exploited in many European, Asian, and African countries due to their production of twins, which are a source of meat, as well as their high milk production, compared to sheep (2). Goats are also characterized by their ability to benefit from all sources of poor-quality feed, such as shrubs and bushes, more than other animals, such as cows or sheep (3), as well as their tolerance to different environments.

The importance of goats in Iraq lies in their adaptability to harsh environmental conditions and the ability to feed on poor fodder. Therefore, goats have

recently received more attention, especially since breeds with a broad scope for genetic improvement will contribute to filling part of the deficit in the lack of meat, milk, and their high prices (2).

The number of goats in Iraq is 1.6 million, according to the Food and Agriculture Organization of the United Nations (4). Scientific and technical advances have led to significant progress in the genetic improvement of farm animals, especially when using molecular indicators to improve many quantitative traits (5).

Several techniques have been used to detect the genetic structures of animal groups, including gel-electrophoresis, polymerase-chain-reaction (PCR) technology, the genetic morphology of single nucleotides or Single-Nucleotide-Polymorphism, and Nuclear DNA (5, 6).

Recently, molecular studies of goats based on mitochondrial DNA sequences (mtDNA) were conducted to investigate the origin and evolution of goats (6).

The *Cytochrome b* (*Cytb*) gene is one of the encoded genes of mtDNA. The mtDNA genes have been widely used in phylogenetic studies due to their ease of access. They are valuable in evolutionary processes and generally follow an inheritance pattern compatible with the recombination-evolutionary construction (6). The *Cytb* gene is one of the genes that have a role in transferring electrons in the respiratory chain and can be identified as a target for evolutionary analysis and species identification (7). In addition, it is unique among the genes containing a genetic code that can encode for a group of proteins at the level of different species so that it can be used to classify animal breeds or determine the genetic relationship between them (7). Several international studies have been conducted using the *Cytb* gene to analyze genetic information, such as the studies conducted on Turkish (8), Indian (7), Iranian (9), and Indonesian goats (10-13). The *Cytb* gene in goats is estimated to be about 1140 bp and encodes 377 amino acids, starting with the ATG codon and ending with the AGA stop codon (14). The *Cytb* gene is also one of the genes least subject to change as it is considered one of the conserved regions with the absence of basic mutations; therefore, it is among the most sensitive regions to be used as a molecular marker or barcode to determine the purity of species (15).

This study aimed to detect the genetic structures of the local Iraqi goats bred in the central and southern regions of the country and investigate the possibility of benefiting from their genetic structures to construct improvement programs for increasing the productivity of these animals.

2. Materials and Methods

2.1. Blood Samples

This study was conducted in the Laboratory of Genetic Engineering, College of Agriculture, University of Basra, Basra, Iraq. Blood samples (15 samples) were collected from the local black goat breed belonging to the College of Agriculture, University of Basra station. A total of 5 ml of each animal's blood were drawn from the jugular vein in the neck after hair removal using a medical syringe with a 10 ml capacity. Afterward, the jugular vein area was sterilized with 70% ethyl alcohol. The blood samples were then placed in tubes containing an anticoagulant substance (Ethylene Diamine Tetra Acetic Acid-EDTA) and kept freezing at -18℃ until the DNA extraction was performed.

2.2. DNA Extraction

DNA was extracted from blood samples of local black goat breeds in the Genetic Engineering Laboratory, College of Agriculture, University of Basra, Basra, Iraq, using an extraction kit supplied by Invitrogen (USA), according to the steps mentioned in the protocol attached to the kit.

2.3. DNA Quantification

The amount and concentration of DNA were measured for each sample by the Nanodrop device (Thermo Fisher Scientific, USA) to know the size of the whole genome (the amount of DNA in ng/µl).

2.4. Polymerase Chain Reaction

The primer for the *Cytb* gene was designed by the BioNeer (Korea), which was used in the experiment, with a size of 670 bp.

The materials for PCR were prepared and placed in a container containing pieces of ice to protect them from heat. Work was done in a sterile and clean place in a particular PCR cabin (PCR Cabinet), which contained UV rays to sterilize micropipettes and tubes. Sterile medical gloves were also used when working. The PCR mixture was prepared in an Eppendorf tube with a capacity of 100 µl, and the final sum of the components was 25 µl. The tubes were then placed in a centrifuge for 30 sec. Table 1 shows the quantities of materials used in the reaction. Table 2 shows the primers used in this technique, and table 3 shows the program for the primers of this technology (16).

Table 1. *Cytb* gene primers

F: 5-AACATCCGAAAGACCCACCC-3 R: 5-TTGGCTGATTGGGCGGAATA-3

Table 2. Chemical materials

Master mix	Primer	DNA Templet	Distill water	Final size
12.5	R-1, F-1	5	5.5	25

Table 3. PCR program

Stages	Tempt (℃)	Time (min)	Number of cycles
Initial Denaturation	94	2:00	1
Denaturation	94	0:30	
Annealing	58	0:30	35
Elongation	72	0:30	
Final Elongation	72	10:00	

2.5. Electrophoresis for the PCR Product

The agarose gel electrophoresis technique was used to determine the success of the DNA amplification process. A DNA marker (100 bp) was placed in the first hole by mixing 1.5 µl of the molecular weight indicator with 3.5 µl of methyl orange dye. As for the rest of the pits, the PCR product was placed in them in an amount of 5 µl. It was then passed over the agarose gel at a concentration of 2% (that is 0.5 g of agarose was dissolved in 25 ml of 1X TBE solution with the addition of 1 µl of ethidium bromide dye). Afterward, the electric current was fixed at 75 volts 65 mA for 40 min, and after the migration was completed, the gel was examined with a data documentation device to see the quality of the packets in the gel.

2.6. Statistical Analysis

2.6.1. Genetic Characterization

2.6.1.1. Assembly and Alignment

Forward and Reverse strand sequences were collected using the Codon Code Aligner software (version 7.1.1, Codon Code Corporation). The results of the *Cytb* gene sequencing were compared to the standard sequences published in the online National Center for Biotechnology Information (NCBI), and the sequences were aligned using the BioEdit software (17).

2.6.1.2. Phylogenetic Tree

The phylogenetic tree was drawn by the Neighbor-joining tree (NJT) method using the MEGA 7.0 program (18). The NJT illustrates the relationships between haplotypes or populations from the genetic distance estimation (19).

2.6.2. Genetic Distance

Genetic distance was calculated using the Arlequin ver software (version 3.5.1.2). It was calculated based on the statistical FST value, which is part of the total variance due to the differences between subpopulations within the total population. An FST value of 0-0.05 means there are low genetic differences, 0.05-0.15 means medium genetic differences, and 0.15-0.25 means high genetic differences. There are very high differences if the FST value is 0.25 or more.

3. Results and Discussion

The results of the electrophoresis using agarose gel for the PCR product for the *Cytb* gene (Figure 1) showed the success of the amplification process with expected sizes (667 bp) resulting from the amplification process.

All studied samples showed success when the amplification was performed to detect the sequences of nitrogenous bases, and the alignment was made using the BioEdit software to make a comparison between the sequences of the black Iraqi goats and some of the

global breeds, whose sequences were obtained from the NCBI gene bank (Table 4).

A comparison of the sequences of nitrogenous bases of the local Iraqi goats to the rest of the international breeds revealed that there was a genetic mutation at the nitrogen base (46) of the *Cytb* gene, which is a substitution mutation from the nitrogen base A>C, where the substitution was from the purine group to the pyrimidine group (Figure 2). This mutation distinguished the black Iraqi goat breed from the other global breeds under comparison.

3.1. Amino Acids Sequence

A total of 223 amino acids were obtained from the studied segment of the *Cytb* gene. The highest percentage was for the amino acids Isoleucine and Leucine, as the ratio reached 14.34% and 11.21%, respectively. While the mutation led to Asparagine's transformation into Histidine, Asparagine acid contained two genetic codes (AAC-AAU). The mutation in the sequence of nitrogenous bases led to the transformation of one of the two codes from (AAC>CAC). It was the only mutation found in this strain (Figure 3).

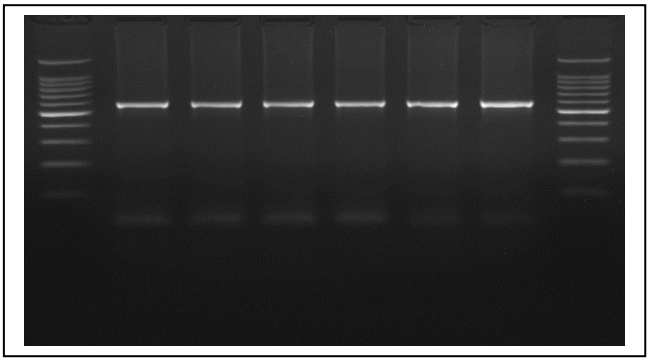

Figure 1. The results of electrophoresis using agarose gel for the PCR product for the Cytochrome b gene

Table 4. The sequences that were compared with the sequences of local Iraqi goats

accession no.	Country	Breed	Year
MN170291.1	India	Andaman Local Goat	2019
MN170286.1	India	Andaman Local Goat	2019
MN170283.1	India	Andaman Local Goat	2019
MH229952.1	Mongolia	Inner Mongo, lia Cashmere Alashan	2018
LS992662.1	Ireland	Ireland goat1	2018
LS992659.1	Ireland	Ireland goat2	2018
MF871517.1	China	China goat	2017
MH165338.1	Iraq	Mariz goat breed	2018
LR884213.1	Ireland	Ireland goat	2020
LR884201.1	Ireland	Ireland goat	2020
MT396986.1	Russia	Karachaev goat	2020

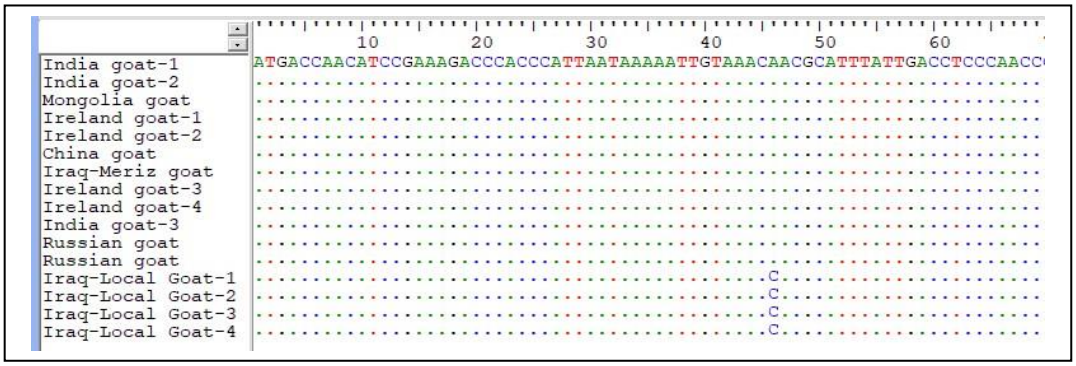

Figure 2. The mutation that occurred in the sequence of the nitrogenous bases of the *Cytb* gene

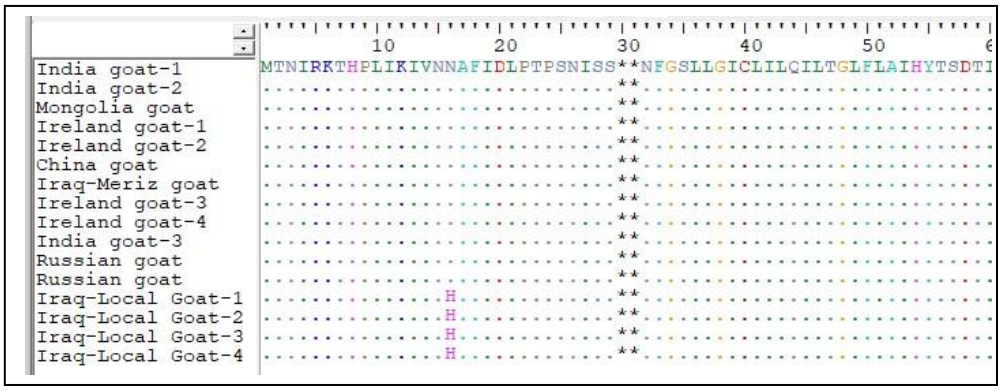

Figure 3. Substitution mutation from Asparagine to histidine

These results did not agree with what was found by Lestari, Purbowati (11) in the Indonesian Kejobong breed, as three mutations were found in the sites (16, 121, and 231). At position 16, Alanine was converted to Threonine, where the G base in the first code of the amino acid Alanine was converted to the A base, changing the genetic code from (GCA>ACA). The mutation also occurred at site 121, where the Alanine was transformed into Threonine, as the genetic code GCG for the amino acid Alanine was transformed into the genetic code ACG. The third mutation at site 231 was the transformation of Alanine into Valine, which resulted from the transformation of the genetic code GCC>GTC.

Most of the mutations in the *Cytb* gene are tandem mutations, such as switching from a purine group to the same group or from a pyrimidine group to the same group, or silent mutations, such as switching from one code to another for the same acid (20). Tamura, Stecher (21) mentioned that most of the synonymous amino acids are the result of the substitution in the nucleotides of the third code, while the non-synonymous amino acids are the result of the replacement of the first or second nucleotide, which confirms what was found in this study.

3.2. Phylogenetic Tree

The results of the phylogenetic tree analysis of the local black Iraqi goats, compared to some international breeds, showed the presence of two main groups. The first group included the black Iraqi goat breed, and the other included the rest of the other global breeds, including the Al-Muraz breed (Figure 4).

The results showed no close genetic relationship between this breed and some global breeds. These results did not match those of Pakpahan, Artama (12), analyzing Hutan Sumatera Indonesian breed genetic tree, compared to other nine Indonesian breeds. The results showed many branches in the phylogenetic tree. Sepehri and Seyedabadi (9) analyzed the phylogenetic tree of the Khalkhali Iranian breed, indicating that this breed shared the same branch with other breeds from Asia and Europe. Jiyanto, Sutopo (10) demonstrated that the Kejobong breed from six different geographic locations exhibited different branches and showed some genetic correlations (22).

These results indicated that the local black Iraqi goat has a unique mother source, compared to other goat breeds worldwide.

3.3. Haplotypes Network

The network of haplotypes showed the presence of two haplotype patterns, one of which included the black Iraqi goat breed, and the other type included all the international breeds under comparison (Chinese, Indian, Russian, Irish, Mongolian, and Iraqi Al-Muraz breed) (Figure 5).

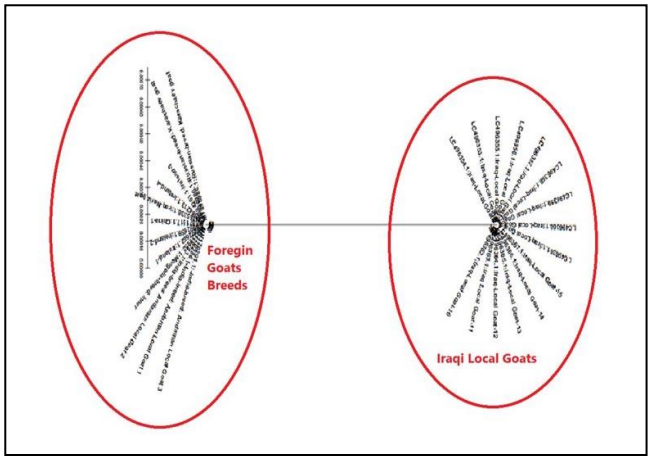

Figure 4. The phylogenetic tree of the local black Iraqi goats in comparison with other breeds

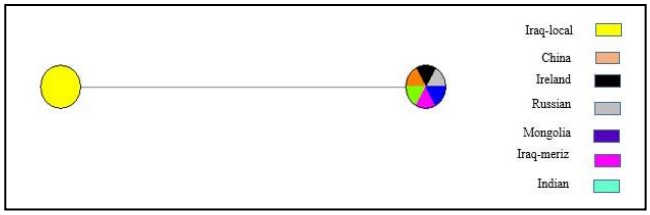

Figure 5. Haplotypes network of local black Iraqi goats in comparison with other breeds

Based on what was stated in previous studies and through the genetic analyses using mtDNA sequences, it was found that most Asian goat breeds, such as Indian, Korean, Indonesian, and Chinese goats, belong to one common ancestor (23). Nicoloso, Bomba (24) also confirmed that domestic goats all over the world are descended from three main ancestors. Sultana, Mannen (25) highlighted the genetic variation of Pakistani goats using mtDNA sequences and concluded that Pakistani goats originated from four different ancestors (A, B, C, D), which might be the four wild breeds. Additionally, Petretto, Dettori (26), analyzing the Italian mtDNA Sarda breed data, confirmed that most of the obtained haplotypes belong to haplogroup A, which is widely prevalent in most continents. Rodionov, Dotsev (27) also indicated that all European goats, as well as Turkish breeds, belong to the haplogroup A and its branches, while some Iranian goat breeds belong to the haplogroups D and G.

The black Iraqi goat breed is unique in its genetic structures, which indicates that Iraq might be the center of this breed's domestication. This breed also showed high genetic diversity, which can be used in genetic improvement programs.

Authors' Contribution

Study concept and design: J. M. O.
Acquisition of data: M. Y. Y.
Analysis and interpretation of data: A. J. A.
Drafting of the manuscript: A. Y. A.
Critical revision of the manuscript for important intellectual content: J. M. O.
Statistical analysis: J. M. O.
Administrative, technical, and material support: M. Y. Y.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article and accepted by the ethics committee of the University of Basrah, Basrah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Mason IL, Mason IL. Evolution of domesticated animals. 1984.
2. Pizarro M, Landi V, Navas González F, León J, Delgado J. Non-parametric analysis of the effects of αS1-casein genotype and parturition non-genetic factors on milk yield and composition in Murciano-Granadina goats. Ital J Anim Sci. 2019;18(1):1021-34.
3. Al-Dabbagh ASS, OramarI RA, editors. A comparative study on milk yield and its composition between Black mountain and Damascus (Shami) goats raised at commercial farms. The 2nd Scientific Agricultural Conference; 2016.
4. Gıda BM, Teşkilatı T. Food and Agriculture Organization of the United Nations (FAO),(2005).
5. Mucha S, Mrode R, Coffey M, Kizilaslan M, Desire S, Conington J. Genome-wide association study of conformation and milk yield in mixed-breed dairy goats. J Dairy Sci. 2018;101(3):2213-25.

6. Wajid A, Hussain T, Wasim M, Babar M, Anjum A, Shah S, et al. The future prospective of genomic biotechnology in animal breeding: their potential for livestock production in Pakistan. J Anim Plant Sci. 2013;23(4):944-55.
7. Petrella G, Pati S, Gagliardi R, Rizzuti A, Mastrorilli P, La Gatta B, et al. Study of proteolysis in river buffalo mozzarella cheese using a proteomics approach. J Dairy Sci. 2015;98(11):7560-72.
8. Kul BC, Ertugrul O. mtDNA diversity and phylogeography of some Turkish native goat breeds. 2011.
9. Sepehri B, Seyedabadi HR, editors. Molecular analysis of Khalkhali goat population based on cytB region of mitochondrial DNA. Biological Forum; 2015: Satya Prakashan.
10. Jiyanto J, Sutopo S, Kurnianto E. The genetic diversity of Kejobong goat based on Cytochrome B gene. J Indones Trop Anim Agric. 2014;39(2):75-82.
11. Lestari DA, Purbowati E, Sutopo S, Kurnianto E. Amino acid sequence based on Cytochrome b gene in Kejobong goat and its genetic relationships among several local goats in Asia. Vet World. 2018;11(8):1196.
12. Pakpahan S, Artama WT, Widayanti R, Suparta I. Genetic characteristics and relationship in different goat populations of Indonesia based on Cytochrome B gene sequences. Asian J Anim Sci. 2016;10:29-38.
13. Lestari D, Sutopo S, Kurnianto E. Amino acid diversity on the basis of cytochrome b gene in Kacang and Ettawa Grade goats. J Indones Trop Anim Agric. 2017;42(3):135-46.
14. Chen S, Ma H, Chen G, Wang L. Complete mitochondrial genome of the Liuyang black goat and its phylogenetic relationship with other Caprinae. Genet Mol Res. 2016;15(2):1-11.
15. Luikart G, Gielly L, Excoffier L, Vigne J-D, Bouvet J, Taberlet P. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proc Natl Acad Sci. 2001;98(10):5927-32.
16. Vakalounakis DJ, Fragkiadakis G. Genetic diversity of Fusarium oxysporum isolates from cucumber: differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. Phytopathology. 1999;89(2):161-8.
17. Hall T, Biosciences I, Carlsbad C. BioEdit: an important software for molecular biology. GERF Bull Biosci. 2011;2(1):60-1.
18. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547.
19. Nei M, Takezaki N. Estimation of genetic distances and phylogenetic trees from DNA analysis. Proc 5th World Cong Genet Appl Livstock Prod. 1983;21(21):405-12.
20. An T, Sun H, Li G, Zhao H, Wong PK. Differences in photoelectrocatalytic inactivation processes between E. coli and its isogenic single gene knockoff mutants: destruction of membrane framework or associated proteins? Appl Catal B: Environ. 2016;188:360-6.
21. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9.
22. Kim J-H, Byun M-J, Ko Y-G, Kim S-W, Kim S-W, Do Y-J, et al. Phylogenetic analysis of Korean native goats based on the mitochondrial cytochrome b gene. J Anim Sci Technol. 2012;54(4):241-6.
23. Zhang S, Gao X, Jiang Y, Shen Y, Xie H, Pan P, et al. Population validation of reproductive gene mutation loci and association with the litter size in Nubian goat. Arch Anim Breed. 2021;64(2):375-86.
24. Nicoloso L, Bomba L, Colli L, Negrini R, Milanesi M, Mazza R, et al. Genetic diversity of Italian goat breeds assessed with a medium-density SNP chip. Genet Sel Evol. 2015;47(1):1-10.
25. Sultana S, Mannen H, Tsuji S. Mitochondrial DNA diversity of Pakistani goats. Anim Genet. 2003;34(6):417-21.
26. Petretto E, Dettori ML, Pazzola M, Manca F, Amills M, Vacca GM. Mitochondrial DNA diversity of the Sardinian local cattle stock. Sci Rep. 2022;12(1):1-7.
27. Rodionov AN, Dotsev AV, Fomenko OY, Bakoev NF, Deniskova TE, Shakhin AV, et al. Complete mitochondrial genomes of Karchaev goat (Capra hircus). Mitochondrial DNA Part B. 2020;5(3):3627-8.