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**REVIEW ARTICLE**

# GENETIC DIVERSITY OF AWASSI SHEEP REARED IN FERTILE CRESCENT BASED ON MICROSATELLITES: A REVIEW

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## ABSTRACT

In general, many domestic breeds of livestock evolved to different geographical regions, Awassi sheep in particular, adapted to Fertile Crescent region where cradle of domestication took place. Awassi sheep has tremendous genetic diversity compared to other domestic sheep breeds of the world. Nowadays, Awassi sheep is in danger of losing their unique and ancient genetic diversity as a result mainly of unwarranted crossing with high-yielding exotic breeds. Another reason for losing diversity is reduction in the population size of Awassi sheep in their origin region of the Fertile Crescent. Unveiling genetic diversity marks the inaugural phase in the execution of breeding and conservation initiatives. Previous literature revealed that microsatellite markers were widely used and accepted until nowadays as useful molecular markers for evaluating genetic diversity of sheep populations along with other markers such as Single Nucleotide Polymorphisms (SNP). Most studies based on microsatellite markers provided information on genetic diversity parameters (e.g. observed and expected heterozygosity, PIC, alleles number, inbreeding coefficient, effective population size, genetic distances values and cluster analyses) within and among Awassi populations. These markers enabled phylogenetic relationships among populations by tree building approach based on genetic distance values. On the other hand, crossbreeding practices are thought to contribute to the genetic erosion of the original Awassi sheep breed, while a reduction in population size is anticipated to have adverse effects on genetic diversity. In this work, we reviewed the microsatellite-based studies which have been published for detecting genetic diversity of Awassi sheep breed in various countries of Fertile Crescent.

***Key words:*** *Genetic diversity, Awassi Sheep, Fertile Crescent, Genetic Markers.*



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## INTRODUCTION

Livestock, in diversified farming systems, perform a critical function by furnishing humans with valuable nutrients through their products. They contribute up to 30-40% of the worldwide agricultural economy (FAO, 2019). Thus, any decline in population size means loss of their genetic diversity and thus loss in their contribution to feeding humanity. Unfortunately, livestock are rapidly losing their genetic resources leading to the loss of two livestock breeds per week (FAO, 2019). The livestock genetic resources should thus, be maintained and documented urgently, and suitable conservation and sustainable breeding plans should be developed, especially in developing nations where technical support and financial availability are limited (Hanotte and Jialin, 2005). In fact, around the world, farm animal breeds of several regions are in danger of becoming extinct because of many threats. Major populations facing such threats are those domesticated sheep breeds (*Ovis aries*) present in the Fertile Crescent (Rashaydeh, 2023). The earliest indication of sheep domestication actually originates from the Fertile Crescent, a specific segment of the Near East. Fertile Crescent, because of its geographical location, has been cradles for civilizations since prehistoric times. Data from several Neolithic human settlements discovered across these areas strongly suggests it was a significant animal domestication hub, primarily for goats and sheep (Zeder, 2008). For that reason, the sheep reared in this important region should be evaluated, reviewed for genetic diversity and phylogenetic relationship. Furthermore, the Fertile Crescent region is famed for its ecological richness, providing a habitat for native livestock breeds that possess distinct traits and excel in adapting to diverse and sometimes harsh environmental conditions. They face risks stemming from reduced profitability, resulting in a decline in population size, along with constraints related to limited water and feed resources. (Polak-Śliwińska *et al*., 2021). Both natural processes and artificial selection have contributed to the formation of the livestock genetic pool in the Fertile Crescent (Groeneveld *et al*., 2010). Nevertheless, one of the major available breeds nowadays is Awassi sheep. For instance, Awassi sheep is reared in all countries of Fertile Crescent for its ability to provide milk and meat in tropical and hard conditions (Toro and Caballero, 2005; Verrier *et al*., 2005). In this regard, Awassi sheep breeds are critical because they meet a large portion of the food demand for people (Galal *et al*., 2008; Suliman *et al*., 2022). Until now, not many studies reviewing biodiversity, characterization, and conservation of Awassi sheep breeds are available (Tapio *et al*., 2010; Rocha *et al*., 2011; Getachew; 2020). The high level of Awassi sheep genetic diversity is needed to address unanticipated future issues. It is considered essential diversity that enables options to satisfy the rising future food demands as well as flexibility to adjust with a wide range of agroecologist (Wollny, 2003).

Different genotyping methods have been developed to evaluate genetic diversity based on different criteria, including heterozygosity, allele frequencies, and inbreeding coefficient (Olschewsky and Hinrichs, 2021). Microsatellite markers (MMs) are widely used, particularly in developing countries. MMs are an effective and cheap tool for assessing genetic diversity and differentiation within and among populations (FAO, 2011). Furthermore, MMs are polymorphic and randomly dispersed across the genomes of livestock species (Karaca *et al*., 2005; Ahmadi *et al*., 2007; Sulaiman *et al*., 2011). They have also been highly informative in establishing connections among and within populations of sheep, characterizing biodiversity, conducting parentage testing, and providing essential insights for the development of appropriate and sustainable sheep breeding programs as well as conservation strategies (Romanov and Weigend, 2001; Rosenberg *et al*., 2001; Al-Atiyat, 2015a; Sheriff and Alemayehu, 2018; Al-Atiyat *et al*., 2018).

However, there has been a scarcity of scientific studies reviewing the biodiversity, characterization, and conservation of Awassi sheep breeds. Therefore, the objective of this review is to shed light on the genetic diversity and phylogenetic relationships among Awassi sheep breeds by synthesizing findings from previous studies conducted across multiple countries using MMs.

**Awassi breed in the fertile crescent:** Sheep farming plays a significant role in the Middle East, as it does around the globe, since it feeds the local populations with milk and meat (Galal *et al*., 2008) In the Fertile Crescent, sheep breeding is mainly focused on the Awassi breed besides other breeds existed as native or crossbreds. Since the milk and meat production of the Awassi breed is low, farmers prefer exotic breeds (Abdullah *et al*., 2002), which has led to a significant decrease in the population size of the breed (Al-Atiyat *et al*., 2012). Today, it is thought that there are around 50 native breeds of sheep in Fertile Crescent including Arab countries and Türkiye (FAO, 1995; ACSAD, 1999) (Table 1).

The most common are Awassi sheep of the fat-tailed sheep. Figure 1 shows images of Awassi breed from different countries of Fertile Crescent. While the indigenous Awassi breed may not match foreign breeds in terms of milk and meat production superiority, their products possess unique qualities preferred by local consumers (Suliman *et al*., 2022). They also have extremely desirable features like adaptability to feed scarcity, diseases and parasite resistance, and tolerance to weather extreme conditions such as high temperature (Galal *et al*., 2008). Also, the Awassi breed contributes significantly to the genetic reservoir of animals, being an integral part of the cultural heritage of the Fertile Crescent region.

Expanding on this, the Awassi sheep breed predominates in the eastern Mediterranean region. It is the most widespread sheep breed in Iraq, Palestine, Syria, the Gulf countries, and the only native breed in Jordan (Al-Atiyat *et al*., 2022). It contributes significantly to sheep breeding in Türkiye (Gürsoy, 2005). Awassi breed in Türkiye has a good contribution to total milk production by local sheep breed (Gürsu and Aygün, 2014). The breed has expanded across all continents, including South America, from its origins in Southwest Asia. It is the only non-European sheep breed that has reached such widespread distribution in the globe (Galal *et al*., 2008). The Awassi breed has been described earlier and more recently in different countries. The Awassi breed is characterized by its long, coarse wool, which adorns the body in a creamy white hue, along with a distinctive convex forehead profile. The breed features a slender, elongated head. The breed presents a bare appearance, often displaying hues of brown or black. Ewes have polled, while rams' horns are normally twisted backwards and downwards with points pointing outwards. The ears are of medium size and commonly hang pendulously. In rams, the neck is generally long and robust, whereas in ewes, the neck is fine. The body is elongated, with medium-length legs set wide apart. The fat-tail is broad, plump, and of moderate length, extending just above the hocks. The tail is formed by a single stem that extends down from the rump and divides into two lobes that widen out near the bottom section before ending abruptly in a short, thick appendix. A huge rift runs through the bottom half of the lobes (Galal *et al*., 2008). An adult Awassi ram typically weighs around 85 kg, whereas an adult ewe averages around 56 kg in body weight (Al-Atiyat *et al*., 2022). An Awassi sheep will typically produce between 60 and 80 liters of milk each lactation, with a modern farming system producing up to

504 liters. According to many researchers, the milk yield of Awassi from different countries ranges from 73 kg to 506 kg (Table 2). The wide range in output of Awassi sheep production can be attributed to several key factors, including selective breeding, lactation duration, birthing method, electrical conductivity, seasonal variations, parity, milking frequency, and production year (Ali *et al*., 2020).

It has been reported that the number of farm animal breeds is declining, because of the intensification of extensive breeding since the middle of the Twenty century due to several reasons such as spread of monoculture and the usage of high-performing animals in breeding on a global scale (FAO, 2019). Eighty-eight percent of farm animal breeds fall under the category of indigenous (sometimes called local or native breeds). This indicates that they are tailored to the local environment and a specific industrial setting (FAO, 2019).

These indigenous breeds collectively contribute to a substantial genetic diversity, particularly notable in developed regions (Zhang *et al*., 2018). According to the FAO (2015), isolated and well-defined populations may have less genetic polymorphism than less-defined populations, which tend to have more genetic variation. In addition, compared to breeds developed locally via farmers' own selection, those developed locally via breeding firms have more variation and be less genetically homogenous (FAO, 2019). Due to the variable levels of genetic variety shown by local breeds, it is crucial to evaluate breed diversity to set priorities and make conservation-related decisions (Boettcher *et al*., 2010). Breed conservation offers protection against future difficulties like the advent of novel and undiagnosed illnesses or the long-term impacts of climate change (FAO, 2019). Therefore, management is also necessary to preserve the genetic variety of many domestic breeds with great historical value.

**Table 1. Native breeds of sheep in Fertile Crescent (Gatenby, 2006; Al-Atiyat *et al*., 2021)**

| Sheep Breeds | Country | Purpose |
|---|---|---|
| Awassi | Jordan, Palestine, Syria, Lebanon, Iraq, Iran and Türkiye | Meat, milk, wool |
| Awassi-Baladi | Jordan | |
| Awassi-Naemi /Naimi | Jordan | Meat, milk, wool |
| Awassi-Saqri | Jordan | Meat, milk, wool |
| Improved Awassi | Palestine | Milk |
| Assaf | Palestine | Milk |
| Najdi | Jordan | Meat, milk |
| Chios | Jordan, Türkiye | Meat, milk |
| Akkaraman | Türkiye | Meat, milk |
| Dağliç | Türkiye | Meat, milk |
| Karaman | Türkiye | Meat, milk |
| Karakul | Türkiye | Meat |
| Tuj | Türkiye | Meat, milk |
| Kıvırcık | Türkiye | Meat |
| Morkaraman | Türkiye | Milk, wool |
| Karayaka | Türkiye | Meat, milk |
| Gokçeada | Türkiye | Meat |
| Hemsin | Türkiye | Meat, milk, wool |
| Karacabey Merino | Türkiye | Meat, milk, wool |
| Anadolu Merino | Türkiye | Meat, milk, wool |
| Sakiz | Türkiye | Meat |
| Imroz | Türkiye | Meat, milk |
| Karadi | Iraq | |
| Hamdani | Iraq | |
| Arabi | Iraq, Iran | Meat |
| Moghani (Shahsavan) | Iran | Meat |
| Loribakhtiyari | Iran | Meat |
| Zel | Iran | Meat |
| Afshari | Iran | Meat |
| Shal or Chal Ghazvin | Iran | Meat |
| Sangsari | Iran | Meat |
| Kordi /Kurdi | Iran | Milk |
| Ghezel | Iran | Milk |
| Karakul Siyah Sarakhs | Iran | Wool |
| Kabode Shiraz (Khakestari Shiraz) | Iran | Wool |
| Zandi Ghom | Iran | Wool |
| Baluchi | Iran | Wool and meat |
| Yazdi | Iran | Wool and meat |
| Kermani | Iran | Wool and meat |
| Sanjabi | Iran | Wool and meat |
| Makoei | Iran | Wool and meat |
| Mehraban Hamadah | Iran | Wool and meat |
| Abidi/Saidi/ Sanabawi | Egypt | |
| Aboudeleik | Egypt | Milk |
| Kanzi / Maenit | Egypt | Meat |
| Barki | Egypt | Meat, milk |
| Barbari | Egypt | Meat |
| Fallahi | Egypt | Meat, milk |
| Sardi | Egypt | Meat, milk |
| Kabashi | Egypt | Milk |
| Ossimi | Egypt | Meat, milk |
| Rahmani | Egypt | Meat, milk |
| Sohagi | Egypt | Meat, milk |
| Farafra | Egypt | |


**Figure 1. Awassi sheep reared in different countries located in Middle East.**

**Table 2. Total milk yield through the lactation for Local and improved Awassi in Fertile Crescent**

| Breed type | Country | Production level | Reference |
|---|---|---|---|
| Local Awassi | Iraq | 73 kg | Al-Samarai and Al-Anbari (2009) |
| | Jordan | 80 kg | Talafha and Ababneh (2011) |
| | Palestine | 150 kg | Rashaydeh *et al.,* (2020) |
| | Syria | 150 kg | Galal *et al.,* (2008) |
| | Türkiye | 121 kg | Biçer *et al.,* (2019) |
| Improved Awassi | Israel | 506 kg | Gootwine (2011) |

**Genetic diversity and its importance in awassi sheep:** The fundamental goal of conservation initiatives, in general, is to maintain as much genetic variation as possible (Hoban *et al*., 2018). In conservation projects, priority should be allocated to breeds with smaller populations and those facing the risk of extinction. Regrettably, the Awassi breed has joined the ranks of indigenous genetic resources in need of protection projects due to their dwindling numbers. In this regard, it is crucial to ascertain the genetic structure of the Awassi breed, which holds significance for the region, and to assess its position among genetic resources. This includes understanding its genetic distance from domestic genotypes and its placement on the phylogenetic tree (Al-Atiyat and Aljumaah, 2014; Al-Atiyat *et al*., 2018). Therefore, identifying the genetic structure of Awassi is essential. Furthermore, molecular approaches such as microsatellite analysis are utilized to uncover conservation priorities in livestock breeds and highlight genetic diversity (Karsli and Fidan, 2019).

**Detection of genetic diversity by microsatellite markers:** MMs, as DNA-based markers, are utilized for precise estimation of genetic diversity. They are molecular markers of DNA sequence that can be tracked for known position on the chromosome. Moreover, they generate information used to quantify links between and within species and breeds (Hoshino *et al*., 2012). Beginning in the 1990s, the introduction of MMs brought about a variety of benefits that went beyond diversity research. MMs consist of small repeat motifs (1-6 bp) and are polymorphic DNA markers. MMs, found in both transcribed and non-transcribed regions of the genome, are highly abundant and prone to mutation, rendering them invaluable for understanding differences within individuals and among populations. (Westman and Kresovich, 1997; Varshney *et al*., 2005). Although MMs motifs are preserved among livestock animals, the number of repeats varies across breeds and individuals within the same species. They are frequently employed to identify genetic diversity due to their ubiquity, random distribution across the genome, ease of accessibility and utilization, high polymorphism, and display of codominant inheritance (Sawicka-Zugaj, 2011).

MMs have long been valued for their role in evaluating genetic diversity and differentiation, both within populations and among them. Thus far, they have been employed in numerous studies on genetic diversity in sheep (Vajed Ebrahimi *et al*., 2017; Al-Atiyat *et al.,* 2018; Karsli *et al*., 2020; Alarslan *et al*., 2021). Their significant polymorphism, characterized by multiple alleles per locus, along with their co-dominant inheritance, has rendered microsatellites highly useful for tasks such as genome mapping, elucidating phylogenetic relationships, and investigating population genetics in livestock. The FAO has identified and suggested the use of several microsatellite markers to characterize the animal genetic resources and to identify the genetic variability as the first strategic priority area (Gaouar, 2016; Ameur *et al*., 2020; Alarslan *et al*., 2021; Badamsuren *et al*., 2021; Xia *et al*., 2021; Loukovitis *et al*., 2023; Ben Sassi-Zaidy *et al*., 2022).

**Awassi sheep genetic diversity assessment through microsatellites:** The recognition of the importance of genetic conservation and biodiversity in local breeds has spurred researchers to explore the genetic diversity among native sheep breeds in the Middle East and other global regions. Awassi sheep was studied because of their important contribution to the livelihood of the people.

Several studies have explored the genetic diversity of Awassi sheep through the utilization of microsatellite markers. Their findings of descriptive statistics for genetic diversity are summarized in Table 3. For instance, in Türkiye, the investigation into genetic diversity encompassed nine indigenous sheep breeds, notably including Awassi, Norduz, Karakas, Cine Caparı, Karayaka, Morkaraman, Karya, Sakız, and Tuj (Yilmaz *et al*., 2014). In the surveyed sheep breeds, a collective count of 501 alleles was recorded, with 154 alleles being identified as unique to specific breeds. The study highlighted that the microsatellite markers employed exhibited more than four alleles, indicating high gene diversity and polymorphic information content (PIC) values at the loci. In another study in Türkiye, three native and two crossbred sheep breeds were investigated using microsatellite markers (Soysal, 2005). In five breeds, a total of 45 alleles were observed, with 174 individuals divided into three groups. Based on the same microsatellite markers (Byrne *et al*., 2004), the genetic diversity levels of five Turkish breeds were compared to European breeds, and it was concluded that the Turkish native breeds have high genetic diversities comparable to Awassi from occupied territories of Palestine, Greece, Cyprus, and Hungary. These results are consistent with the finding that, according to Soysal *et al.* (2005), the Awassi sheep from Türkiye and Occupied territories of Palestine have more alleles per microsatellites than the Icelandic, German, French, and Hungarian sheep breeds. The high genetic diversity observed among the breeds serves as supporting evidence for their proximity to the domestication center (Loftus *et al*., 1999; Bruford *et al*., 2003). In the most extensive microsatellite study conducted on native Turkish sheep breeds, a sample of 594 sheep was collected, and analysis was conducted using 29 microsatellite loci. (Ozmen *et al*., 2020). In this study, a total of 815 alleles were identified, with 28 microsatellite loci demonstrating high informativeness. The results pointed out the absence of significant bottlenecks among the breeds, highlighting the Awassi breed's distinctiveness within the population.

**Table 3. Genetic diversity parameters in Awassi Sheep breeds**

| Region | Ho | He | Na | PIC | $F_{IS}$ | References |
|---|---|---|---|---|---|---|
| Türkiye | 0.23 | 0.66 | 8.17 | 0.80 | 0.66 | Ozmen *et al.* (2020) |
| | 0.72 | 0.82 | 11.39 | 0.87 | 0.14 | Yilmaz *et al.* (2014) |
| | 0.73 | 0.74 | 8.67 | - | 0.01 | Soysal *et al.* (2005) |
| | 0.67 | 0.75 | 6.69 | - | 0.10 | Elbeltagy *et al.* (2015) |
| | 0.58 | 0.75 | 9.25 | - | 0.09 | Doğan (2009) |
| Jordan | 0.67 | 0.70 | 6.17 | 0.65 | - | Al-Atiyat *et al*. (2012) |
| | 0.67 | 0.73 | 12.67 | - | 0.07 | Al-Atiyat and Aljumaah (2014) |
| | 0.67 | 0.76 | - | 0.65 | 0.13 | Al-Atiyat (2015a) |
| | 0.90 | 0.70 | 3.5 | 0.60 | - | Al-Atiyat *et al.* (2014) |
| | - | 0.80 | 5.4 | - | 0.05 | Al-Atiyat *et al*. (2018) |
| Saudi Arabia | - | 0.74 | 8.8 | - | 0.07 | Al-Atiyat *et al*. (2018) |
| Egypt | 0.72 | 0.79 | 10.92 | - | 0.09 | Elbeltagy *et al.* (2015) |
| | 0.66 | 0.70 | 6.54 | - | 0.05 | Othman *et al.* (2016) |
| Syria | 0.70 | 0.74 | 7.08 | - | 0.04 | Elbeltagy *et al.* (2015) |
| Iraq | 0.61 | 0.76 | 3.54 | 0.56 | 0.46 | Mohammed *et al.* (1992) |
| | 0.50 | 0.65 | - | - | 0.22 | Al-Mohamadawi and Ayied (2021) |

Ho: Mean observed heterozygosity; He: Mean expected heterozygosity; Na: mean number of alleles; PIC: Polymorphism information content; Fis: Inbreeding coefficient.

The genetic diversity of Awassi sheep has been thoroughly investigated in Jordan (Al-Atiyat *et al*., 2012; 2014; 2015b). The breeds displayed high genetic diversity, gene flow and effective selective mating, with the studied populations found to adhere to Hardy-Weinberg Equilibrium (HWE). Genetic conservation suggests prioritizing the preservation of genetic diversity within small flocks, especially in contexts devoid of external gene flow (Al-Atiyat, 2012). However, in another study of Jordan Awassi sheep, it was found that all loci were significantly different from HWE, and all sheep had more inbreeding values than expected (Al-Atiyat *et al*., 2014). After reviewing Jordan Awassi sheep, they can be classified into four main genetic groups originating from various geographical locations (Al-Atiyat *et al*., 2014). On the other side, a distinct genetic diversity emerged Awassi and Merino sheep (Al-Atiyat *et al*., 2015). The authors emphasized that the Awassi breed exhibited the highest degree of variability compared to Australian Merino sheep. All these studies collectively indicate that microsatellites represent a valuable genetic tool for sheep population genetics. The results obtained can inform future strategies for managing genetic conservation, particularly in the face of challenges like climate change and crossbreeding restrictions. The five sheep breeds (Harri, Najdi, Naemi (Saudi Awassi), Arb, and Rufidi) reared in Saudi Arabia and Awassi sheep from Jordan were studied to detect the genetic structure and diversity (Al-Atiyat *et al*., 2018). The authors found that sheep reared in Saudi Arabia showed much genetic diversity according to the values of allele numbers, allelic richness and He. The Naemi (Saudi Awassi) and Awassi breeds were found similar genetic structure while both breeds were grouped differently from other Arabian sheep breeds according to structure analyses (Figure 2).

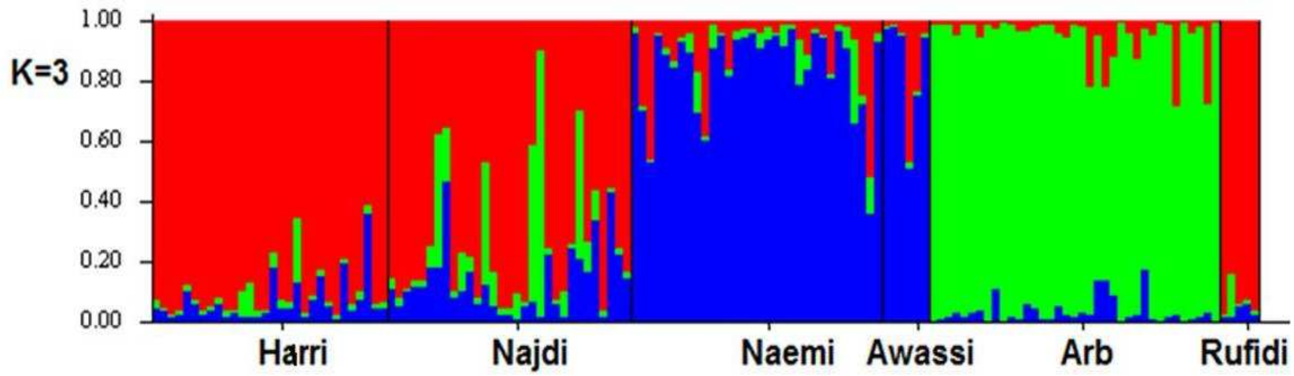


**Figure 2. Genetic differentiation of Awassi sheep and other breeds in Saudi Arabia according to Structure analyses (Al-Atiyat *et al*., 2018).**

Genetic diversity levels within and between populations were investigated in a cohort of 289 animals, employing 13 microsatellites from six native sheep populations (Barki, Farafra, Ossimi, Rahmani, Saidi, and Souhagi) in Egypt. Additionally, 119 Awassi sheep from Egypt, Türkiye, and Syria were included in the study (Elbeltagy *et al.*, 2015). The authors found 229 different alleles, 44 of which were private. The findings of the study unveiled a tapestry of genetic divergence among Egyptian populations, alongside a distinct separation between Awassi populations from Egypt and those from Syria and Türkiye. This intricate web of differentiation hints at the influence of reproductive isolation. The Awassi sheep from Egypt, Syria and Türkiye were grouped differently from other sheep breeds according to Structure analyses (Figure 3).

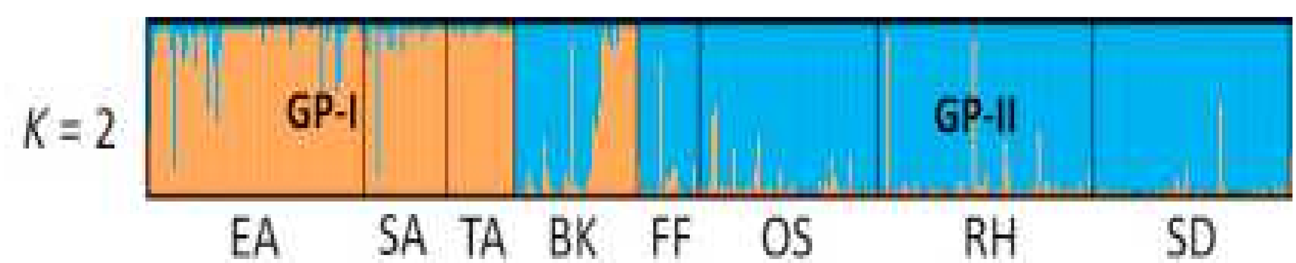


**Figure 3. Genetic differentiation of Awassi sheep reared in different countries and other breeds in Egypt according to Structure analyses (Elbeltagy *et al*., 2015).**
(EA: Awassi-Egypt; SA: Awassi-Syria; TA: Awassi-Türkiye; BK: Barki; FF: Farafra; OS: Ossimi; RH: Rahmani; SD: Saidi).

The Awassi and Naemi breeds were compared in Iraq by using microsatellite markers (Hadi *et al.*, 2020). The authors found that Awassi breed had more alleles than Naemi breed. The authors highlighted that the genetic markers utilized in this research could distinguish between the analyzed breeds and individuals within the same breed, indicating genetic diversity. In another study in Iraq, MMs were used to identify the multiple genetic polymorphisms in the Arabi and Awassi sheep breeds (Al-Mohamadawi and Ayied, 2021). A total of 13 and 19 alleles were found in Arabi and Awassi breeds, respectively. The authors underscored that heterozygote deficiency might stem from the interbreeding of multiple breeds, with population subdivision driven by null alleles, genetic drift and inbreeding further exacerbating this deficit.

**Estimation of observed (Ho) and expected (He) heterozygosities:** The literature suggests that heterozygosity estimates with values greater than 0.5 are considered suitable for genetic diversity studies. in literature heterozygosity estimates surpassing the 0.5 threshold emerge as suitable for genetic diversity researches.

The observed heterozygosity, as well as the expected heterozygosity, estimates observed in Awassi sheep populations in Türkiye, Jordan, Saudi Arabia, Egypt, Syria, and Iraq are relatively high. This leads to the conclusion that the studied sheep populations exhibit a significant degree of genetic diversity within their respective populations (Table 3).

**Estimation of MNAL** Assessing genetic polymorphism within a population, the mean number of alleles emerges as a crucial metric(Halima *et al*., 2012). The significance of the mean number of alleles is contingent upon the population's sample size, considering the potential presence of unique alleles occurring at low frequencies (Qwabe, 2011). As the population size increases, the number of detected alleles may also rise, with a higher count of alleles indicating a greater level of genetic variation (Nei, 1987).

In Table 3, the mean number of alleles, acting as an indicator of genetic polymorphism within the analyzed microsatellites, has been recorded across various populations of Awassi sheep. Encouraging estimates of the mean number of alleles (MNAs) were reported in Table 3. A higher count of alleles implies increased genetic variation (Nei, 1987), making it a key consideration in conservation programs. While these reports underscore the presence of high polymorphism, it's essential to recognize that the mean number of alleles is contingent on the sample size. Generally, the number of observed alleles tends to increase as the population size expands (Al-Atiyat *et al*., 2014).

Hence, it becomes imperative to sample populations of relatively equal sizes to ensure accurate comparisons (Qwabe, 2011). Nevertheless, certain studies have chosen remarkably small sample sizes, deviating significantly from the FAO's recommended approach regarding microsatellite marker analysis (FAO, 2011). This disparity may introduce bias when estimating genetic parameters like HWE and MNA, potentially compromising the accuracy of the findings.

It's also worth noting that the majority of sheep genetic diversity studies (as indicated in Table 3) utilized a restricted number of microsatellite markers. Achieving the utilization of all 30 microsatellites, as recommended by FAO (2011), was only reported in the study by Ozmen *et al.* (2020). Performing genetic diversity studies with an expanded repertoire of microsatellite markers not only yields richer insights into population structures but also facilitates comparisons with findings from prior studies utilizing diverse marker subsets (FAO, 2011).

**Estimation of polymorphic information content:** Polymorphism information content (PIC) is a metric derived from both the total number of alleles and their distribution frequencies within the population. It provides information about how polymorphic a genetic marker is and the extent of variation at the locus under study. In other words, it provides information about how useful the marker is at the locus under study. A PIC value above 0.50 indicates that the marker provides a high level of information. A PIC value higher than 0.75 indicates that the locus is informative at much higher levels and is very useful for genetic variation and genetic mapping studies (Botstein *et al*., 1980). Aligned with this observation, the majority of studied sheep populations utilized highly polymorphic markers, as indicated in Table 3. The Polymorphic Information Content (PIC) is shaped by both heterozygosity and the allele count (Al-Atiyat *et al.*, 2014), establishing microsatellite markers as the prime choice for genetic characterization and diversity investigations.

**Level of inbreeding ($F_{IS}$):** Individuals descended from common ancestors are called inbreeding, and the mating of related individuals is called inbreeding. As a result of inbreeding, populations deviate from Hardy-Weinberg equilibrium. The reason for this deviation because of inbreeding is the heterozygote deficiency in the population or in other words homozygote excess. $F_{IS}$ value varies between -1 and +1. Negative values indicate heterozygosity while positive values indicate homozygosity excess. In large randomly mating populations, the Fis value is expected to be zero or negative. Given this background information, various scholars have reported moderate to high levels of inbreeding in Awassi sheep populations; for instance, Awassi sheep breeds of Turkiye, ($F_{IS}$ = 0.66) (Ozmen *et al*., 2020), ($F_{IS}$ = 0.14) (Yilmaz *et al.*, 2014), ($F_{IS}$ = 0.10) (Elbeltagy *et al*., 2015). Furthermore, $F_{IS}$ values of some Awassi sheep breeds were reported with wide range. For example, Awassi sheep of Jordan was $F_{IS}$ = 0.13) (Al-

Atiyat *et al*. 2015a) and Awassi sheep breeds of Iraq, ($F_{IS}$ = 0.22-0.46) (Mohammed *et al*., 1992; Al-Mohamadawi and Ayied, 2021). The broad spectrum observed could stem from factors such as the modest size of the sheep population, a closed breeding system, and the utilization of a restricted number of breeding rams over successive years. Further insights of FIS estimates might be related with the heterozygosity and mean number of alleles (MNA) estimates presented in Table 3.

However, acceptable mean values of $F_{IS}$ were reported, including 0.01 for Turkish Awassi sheep (Soysal *et al*., 2005), 0.09 for Turkish Awassi sheep (Doğan, 2009), 0.05 and 0.07 for Jordan Awassi sheep (Al-Atiyat *et al*., 2014; 2018), 0.05 (Othman *et al*., 2016), 0.09 (Elbeltagy *et al*., 2015) for Egyptian Awassi sheep, 0.07 for Saudi Awassi sheep (Al-Atiyat *et al*., 2018), and 0.04 for Syria Awassi sheep (Elbeltagy *et al*., 2015). These intermediate levels of inbreeding might stem from the practice of moderately related individuals engaging in mating.

**Phylogenetic relationships among Awassi Sheep:** Microsatellite markers are useful for identifying genetic diversity and establishing evolutionary relationships among different animal breeds. The genetic distance, factorial correspondence analysis (FCA), and structural analyses have been used to examine the phylogenetic connections between different breeds of sheep (Raheem Alnajm *et al.,* 2021), chickens (Karsli *et al*., 2019), goats (Ceccobelli *et al*., 2020), and cattle (Demir and Balcioglu, 2019). This section discussed microsatellite-based studies on the evolution of Awassi sheep breeds.

Charting the evolutionary trajectory of populations has risen to the forefront as a pivotal domain within molecular evolution. To assess the evolutionary relationships between sheep breeds, earlier studies utilizing microsatellite markers were used. In several studies, phylogenetic trees for the Awassi sheep breeds were created using genetic distance values. Al-Atiyat *et al*. (2018) and Raheem Alnajm *et al*. (2021) showed that the Awassi and Naemi (Saudi Awassi) were more closely related, while Al-Atiyat *et al*. (2021) showed that the Blackface Awassi and Black Najdi were more closely related than other sheep breeds. Also, Harri individuals showed genetic closeness to Awassi (Adam *et al*., 2015). The Awassi and Morkaraman breeds belong to the same group in the evolutionary tree (Yilmaz *et al*., 2014). Therefore, it is unsurprising that both the Awassi and Morkaraman breeds were raised in Eastern Türkiye. Conversely, the phylogenetic tree displayed a distinct separation between Awassi sheep and exotic breeds such as Merino flocks, as well as all other Turkish sheep, consistent with their respective breed origins (Ozdemir *et al*., 2011; Al-Atiyat, 2015b; Ozmen *et al*., 2020) (Figure 4). These results are consistent with what is known about the history of these populations, which includes their remote locations and the long history of evolution of their common ancestors. A previous study reported a significant differentiation between Awassi sheep and the Merino Spanish breed according to genetic distance values (Arranz *et al*., 2001).

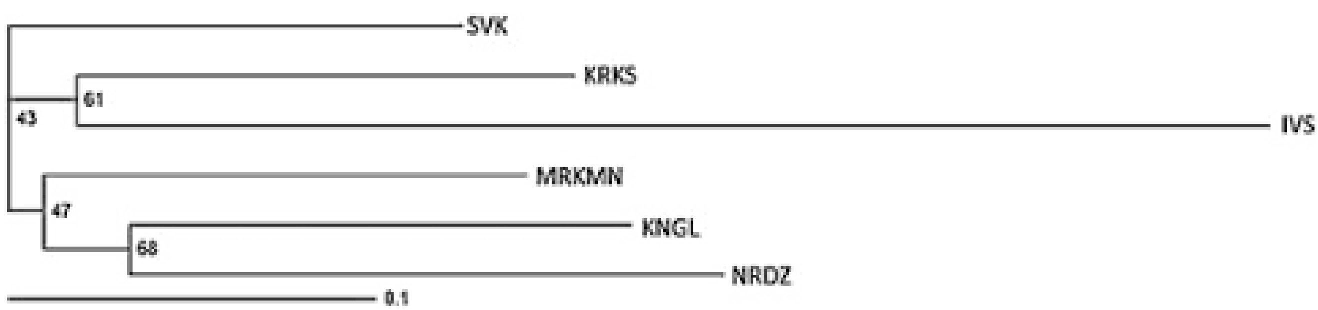


**Figure 4. Neighbour-joining tree constructed using Nei's genetic distance (DA) for Savak (SVK), Karakas (KRKS), Kangal-Akkaraman (KNGL), Norduz (NRDZ), Morkaraman (MRKMN) and Awassi (IVS) sheep breeds (Ozmen *et al*., 2020).**

FCA is an analysis for locating breeds in three dimensions and exposing breeds' evolutionary relationships. The FCA analysis in many studies revealed that the Awassi sheep breeds were grouped individually. (Al-Atiyat *et al*., 2012, 2014; Ozmen *et al*., 2020; Arranz *et al*., 2001; Ozdemir *et al*., 2011). Awassi sheep populations are distinctly separated from all other Turkish sheep breeds (Karakas, Akkaraman, Morkaraman, Kangal-Akkaraman, and Norduz) (Ozmen *et al*., 2020) (Figure 5). On the contrary, Yilmaz *et al*. (2014) reported that Awassi sheep and Morkaraman showed up in the same group according to FCA analysis. Furthermore, Al-Atiyat *et al*. (2018) and Raheem Alnajm *et al*. (2021) reported that Awassi and Naemi sheep belong to the same group according to FCA analysis.

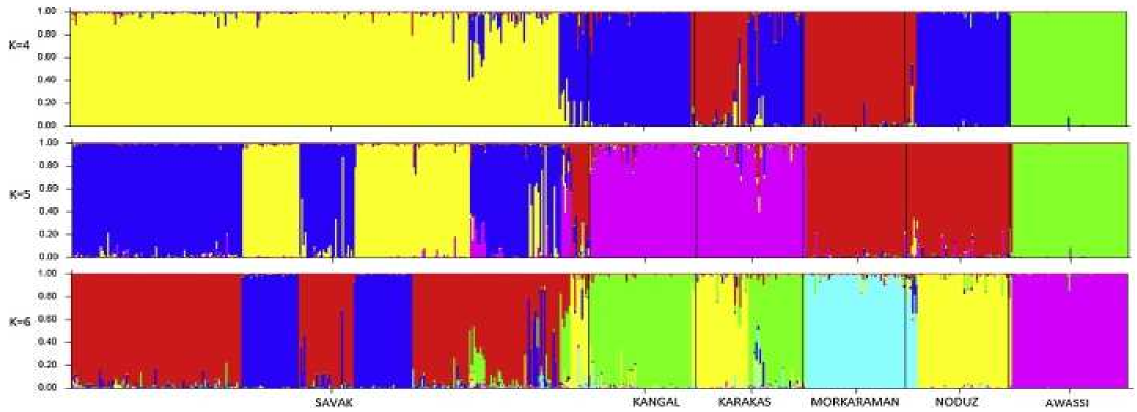


**Figure 5. Genetic differentiation of Awassi sheep and other breeds in Türkiye according to Structure analyses (Ozmen *et al*., 2020).**

**Conclusion And Future Goals:** Breed priority, conservation, and management decisions are based on the traits of genetic variation, which can be better understood by making and using DNA-based techniques. MMs were the most common, with a 48% use rate at least up to time between 2016 and 2020. On this basis we reviewed the current microsatellite-based studies of Awassi sheep, the most common breed, in the Fertile Crescent. It was found Awassi sheep breeds exhibit a notably high level of genetic diversity compared to other local sheep breeds raised in the Fertile Crescent and elsewhere around the globe. However, today, it is seen that there are still challenges in the genetic diversity of the Awassi breed in some regions related somehow with crossing practices with high-yielding exotic breedsSo that Awassi breeds are facing threats of losing their unique genetic resource and traits, which are important for their tolerating harsh local environment and for sustainable use in the future. We recommend the initiation of sustainable breeding programs and the implementation of policies addressing the choice between purebred or crossbred approaches, which are imperative measures for averting genetic erosion in Awassi sheep. Researchers should also keep use MMs along with new molecular techniques of next-generation sequencing (SNP and WGS) to find genetic diversity in a larger part of sheep genome.

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