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Microsatellite Analysis for Genetic Polymorphism and Parentage Confirmation of Arabian Horse

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Abstract

THE INTENT of the research was to use microsatellite DNA typing to create a parentage testing method for Arabian horses. A large dataset of 100 animals was genotyped, including 20 mares for individual identification, 7 stallions, and 73 foals for parentage testing. Blood samples were used to extract genomic DNA, which was then examined using 14 microsatellite markers (VHL20, HTG4, AHT4, HMS7, ASB17, HTG6, AHT5, ASB23, HMS6, ASB2, HTG10, HTG7, HMS3, and HMS2). Multiplexing PCR was used as the genotyping technique, and it showed effective amplification across all targeted loci. A mean value of 6.36 was found in Arabian horses, with a range of 4 to 8 alleles per locus. The 14 microsatellite loci had a total exclusion probability of 0.9998 with expected heterozygosity ranging from 0.548 to 0.831 (mean 0.699). Interestingly, the loci for ASB2, ASB17, ASB23, HMS7, and HTG10 showed comparatively increased polymorphic information content (PIC) values (> 0.7). The Mendelian compatibility criteria were used to qualify the foals, confirming the accuracy of the DNA typing technique for Arabian horse identification and parentage confirmation. These results highlight the great potential of microsatellite DNA typing to improve pedigree management and verification in breeding operations for Arabian horses.

Keywords: Microsatellite, Arabian horses, Polymorphism, Parentage verification.

Introduction

Arabian horses are regarded as a type of the world's oldest and most significant horse breeds. They are instantly identified by their particular beauty and look, but they also feature several functional and structural adaptations for increased athletic performance, and their amazing endurance is well known across the world [1].

The implementation of parentage testing techniques by breed registry authorities around the world is necessary to guarantee the integrity of horse pedigrees, which is essential for breeding management plans. For such purposes, co-dominant genetic markers that are inherited according to Mendelian principles work well. DNA testing methods, which are now widely available and gradually being used by the majority of ethical laboratories, can greatly increase the success of paternity testing. These tests are a great alternative to more traditional methods. They are appealing because they may be highly automated, need little amounts of biological material, function with non-fresh blood samples, and have simpler, less costly procedures [2].

Relations for genetic evaluation, conflict decision in the breeding animal trade, and fundamental parent confirmation in large-scale production systems are now more dependable due to the ease with which DNA markers have made it possible to confirm possible parents. For the past 20 years, this verification has been based on short tandem repeat markers (STR), referred to as microsatellites. They are very polymorphic, therefore even with a small number of markers, individual differentiation is possible. Simple exclusion can be utilized to

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ascertain parentage when using STR because of their highly polymorphic nature [3].

Molecular genetic techniques validate registries, establish familial relatedness, and verify identity in the majority of farmed animals [4]. Genetic markers such as microsatellites and short tandem repeats (STR) are commonly used in paternity exaination and genetic variability researches [5-7]. Combining multiplex PCR with the electrophoresis of fluorescently labeled PCR products has improved the use of microsatellites for genetic diversity estimating, genetic maps structures, quantitative trait loci mapping, horse parentage testing and pedigree recording [8].

Microsatellite DNA typing used by horse registries to confirm pedigree records and answer parentage queries [9]. In reality, horse breeders give breeding societies the parentage information about their horses, and the societies enter the information into the register to create pedigrees. DNA genotyping technology is one of the most dependable and effective methods for creating and analyzing pedigrees. Because the cost of reagents and instruments has decreased, DNA genotyping is currently the most efficient way to maintain animal pedigrees in large populations [10].

STRs make it possible to use hair samples instead of blood samples, which was not possible with previous blood typing methods. Furthermore, according to Luis et al. [11]. The genetic makeup and pedigree testing of various horse breeds have been extensively studied using STR loci [12-16] and STRs have been effectively applied to the study of small populations of closely bred animals [17].

Utilizing DNA typing that has been standardized by frequent comparison testing, pedigree control for a variety of animal species has been conducted regularly in most countries with the guidance of the International Society for Animal Genetics (ISAG) [18]. Characterising the genetic polymorphism of 14 microsatellite markers and evaluating their suitability and effectiveness for use in Arabian horse parentage verification were the objectives of this study.

Materials and Methods

Animal Welfare and Ethical Statement

All experimental procedures were reviewed and approved by the Animal Research Ethics Committee, Zagazig University, Egypt.

Sample collection and DNA extraction

Whole blood samples obtained from 100 Arabian horses (20 mares, 7 stallions, and 73 foals) reared in El-Zahra Station, Egypt. Following the manufacturer's instructions, genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Cat. No. 69504, Qiagen). A 1% agarose gel electrophoresis and a QuantiFluor dsDNA System were used to assess the extracted DNA's purity and concentration, respectively.

Microsatellite fragment analysis and parentage testing

A total of 14 microsatellites were chosen for this investigation (Table 1). According to the ISAG's horse applied genetics committee, these microsatellite markers can be used to verify the parentage and identify Arabian horses individually.

Utilizing fluorescently labeled primers, microsatellite markers were mixed in a multiplex PCR reaction and amplified in a total volume of 15 µl of the following mixture: 1 µl of genomic DNA (20 ng), 0.2 µl of Smart Taq HotStart DNA polymerase (Applied Biosystems, USA), 1.8 µl of primer mix, and 12 µl of PCR master mix. The PCR amplification protocol was as follows: initial denaturation for 10 minutes at 96°C was followed by 32X at 96°C for 30 seconds, 60°C for 40 seconds, and 72°C for 60 seconds, with a final extension step of 72°C for 10 minutes following this. A T100-thermal cycler (Bio-Rad, USA) was used to conduct multiplex PCRs.

Following the suggested procedures, PCR products were denatured with formamide and electrophoresed on an ABI PRISM 3500 xl Genetic Analyzer (Applied Biosystems, USA). Applied Biosystems' GeneMapper® version 3.0 software was used to evaluate the genotyping data, and the GeneScanTM-500 LIZ®, an internal size standard, was used to determine the fragment size. Parentage testing was conducted in accordance with ISAG criteria for the current DNA type and Mendelian inheritance.

Handling data statistically

The data were firstly examined in Microsoft Office Excel 2007, to avoid errors, and then converted into input files for further analyses. The alleles number per locus (k), observed and expected heterozygosity (HObs and HExp, respectively), polymorphic information content (PIC), non-exclusion probabilities were calculated [19], and Hardy–Weinberg equilibrium (HWE), was estimated using Cervus version 3.0.3.

The expected heterozygosity according to Hardy-Weinberg [20] is

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

Where, *pi* is the *i*th allele frequency.

The polymorphic information content (PIC) [21].

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where Pij is the number of the jth allele for marker i for n alleles.

The combined exclusion probability

$$p = 1 - \prod_{i=1}^{n}(1 - p_i).$$

Where n is the number of markers, Pi is individual exclusion probability for one marker, Π= Symbol of product, and P = combined exclusion probability[22].

Results

In this research, 14 microsatellite loci were successfully amplified with PCR and separated by capillary electrophoresis. Genotypes of each marker locus were determined by fragment analysis (Fig. 1).

In the Arabian horse population investigated, microsatellite markers exhibited notable polymorphism, as evidenced by Tables 2 and 3. The allelic diversity ranged from 4 (HTG4) to 8 (HTG10, HMS2), with an average of 5.71 alleles per locus observed in this study (Table 2). Both observed and expected heterozygosity values varied from 0.418 to 0.776 (mean 0.597) and from 0.486 to 0.777 (mean 0.646), respectively. The polymorphic information content (PIC) ranged from 0.394 to 0.738, with a mean value of 0.600. Hardy-Weinberg equilibrium was found to be non-significant in most of the loci (Table 3).

Combined non-exclusion probability was calculated in the presence of two parents (NE-1P) and one parent (NE-2P) (Table 3). NE-1P values varied between 0.618 (VHL20) and 0.883 (HTG7). The lowest NE-2P value (0.439) was observed in VHL20, and the highest NE-2P value (0.787) was determined in the HTG7 locus. The total exclusion probability of 0.9999 across the 14 microsatellite loci was obtained (Table 3).

The fourteen microsatellite markers were compatible with Mendelian inheritance for parentage confirmation in this DNA typing (Table 4). There are complete match between foal, sire and dam across 14 microsatellite markers, where the foal inherit one allele from sire and dam along 14 markers used in the study.

Discussion

Microsatellite type is extensively utilized in the horse breeding industry across several nations for the purposes of identifying individuals, controlling parentage, and settling disputes regarding maternity or paternity. Moreover, investigations of parentage, kinship, and reproductive success have used DNA markers to demonstrate a wide range of individual differentiation [23].

Horse microsatellites were initially known by [24] and [15], where they discovered a set of (CA)n repeats and proved that horses were extremely polymorphic. The accuracy and specificity of DNA-based methods make them potentially superior to conventional parentage testing methods in a number of ways.

Microsatellites are the favored markers increased polymorphism and ease of scoring by a computer program. According to [25], this implies that microsatellite alleles were correctly transmitted down to the next generation and thus semi-automated DNA type analysis is feasible.

With its use of microsatellite DNA markers for precise confirmation, this work marks a substantial breakthrough in parentage testing in Arabian horse populations. In order to preserve pedigree integrity and improve breeding operations, the research develops a reliable technique for individual identification and parentage verification by a careful examination of 14 microsatellite loci. The results illustrate the efficacy of microsatellite DNA typing in Arabian horses, with encouraging results.

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The informativeness of a locus based on the number of allele, so different parameters such as heterozygosity (Ho) and probability of exclusion values were used and detected by the allele frequencies distribution in populations [26, 27].

Determining the cumulative power of exclusion (PE) across all 14 microsatellite loci-which was determined to be 0.9999 in the Arabian horse population—is a noteworthy result of this study. This exceeds the 0.9995 threshold set by the International Stud Book Committee (ISBC), suggesting that the microsatellite marker method is theoretically suitable for reliable parentage verification. Strong proof of the correctness and dependability of the DNA typing technique used in this investigation is provided by such a high PE value.

The ISBC has required an increased probability of exclusion (PE) value for the purpose of verifying the identity and parentage of horses [9]. Molecular markers for pedigree verification are the most widely used use of PE, a parameter to address problems with certain population genetic markers [28].

The 14 microsatellite loci were used for the parentage testing, which showed Mendelian compatibility. This confirms that the DNA typing method is a reliable way to identify Arabian horses individually and verify their paternity. The study guarantees the legitimacy and precision of the parentage results by following Mendelian inheritance patterns, giving the genetic analysis carried out credibility.

The results of this study have important ramifications for breeding programs for Arabian horses, especially in Saudi Arabia and other areas where maintaining the integrity and purity of Arabian horse pedigrees is important. Establishing a strong microsatellite DNA typing-based parentage testing system allows breeders and regulatory agencies to efficiently confirm parentage, identify mistakes, and stop unethical breeding activities. Additionally, the high informativeness of the microsatellite markers found in this study offers useful genetic data for keeping genetic difference in the Arabian horse population and improving breeding tactics.

Conclusion

Using 14 microsatellite DNA markers, the study is an extensive attempt to create and validate a parentage examination method for Arabian horses. The outcomes show that microsatellite DNA typing is a reliable method for confirming individual identification and parentage in the Arabian horse population. High polymorphic variety, cumulative power of exclusion, and Mendelian compatibility make the suggested method a dependable and useful way to improve pedigree management and verification in Arabian horse breeding operations. This study lays the groundwork for additional research aimed at improving genetic analysis in equine populations and improving parentage testing procedures.

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Conflict of interest
The authors declare no conflict of interest.

TABLE 1. Locus names, Primer sequences of the 14 microsatellite loci

Locus	Primer sequence (5'→3')	Dye	Reference
AHT4	F-AACCGCCTGAGCAAGGAAGT R-GCTCCCAGAGAGTTTACCCT	FAM	Binns *et al.* (1995)
AHT5	F-ACGGACACATCCCTGCCTGC R-GCAGGCTAAGGGGGCTCAGC	VIC	Binns *et al.* (1995)
ASB2	F-CCACTAAGTGTCGTTTCAGAAGG R-CACAACTGAGTTCTCTGATAGG	VIC	Breen *et al.* (1997)
ASB17	F-GAGGGCGGTACCTTTGTACC R-ACCAGTCAGGATCTCCACCG	PET	Breen *et al.* (1997)
ASB23	F-GCAAGGATGAAGAGGGCAGC R-CTGGTGGGTTAGATGAGAAGTC	VIC	Irvin *et al.* (1998)
HMS2	F-CTTGCAGTCGAATGTGTATTAAAT R-ACGGTGGCAACTGCCAAGGAAG	TAM	Guerin *et al.* (1994)
HMS3	F-CCAACTCTTTGTCACATAACAAGA R-CCATCCTCACTTTTTCACTTTGTT	NED	Guerin *et al.* (1994)
HMS6	F-GAAGCTGCCAGTATTCAACCATTG R-CTCCATCTTGTGAAGTGTAACTCA	JOE	Guerin *et al.* (1994)
HMS7	F-CAGGAAACTCATGTTGATACCATC R-TGTTGTTGAAACATACCTTGACTGT	FAM	Guerin *et al.* (1994)
HTG4	F-CTATCTCAGTCTTGATTGCAGGAC R-CTCCCTCCCTCCCTCTGTTCTC	FAM	Ellegren *et al.* (1992)
HTG6	F-GAAGCTGCCAGTATTCAACCATTG R-CTCCATCTTGTGAAGTGTAACTCA	JOE	Ellegren *et al.* (1992)
HTG7	F-CCTGAAGCAGAACATCCCTCCTTG R-ATAAAGTGTCTGGGCAGAGCTGCT	TAM	Ellegren *et al.* (1992)
HTG10	F-CAATTCCCGCCCCACCCCCGGCA R-TTTTTATTCTGATCTGTCACATTT	NED	Marklund *et al.* (1994)
VHL20	F-CAAGTCCTCTTACTTGAAGACTAG R-AACTCAGGGAGAATCTTCCTCAG	FAM	van Haeringen *et al.* (1994)

TABLE 2. Allele frequencies of 14 microsatellite polymorphisms in the Arabian horse

Locus	No. allele	Allele (frequency)							
VHL20	7	I (0.155)	L (0.270)	M (0.060)	N (0.170)	O (0.005)	P (0.030)	R (0.310)	
HTG4	4	K (0.235)	L (0.130)	M (0.620)	N (0.015)				
AHT4	6	H (0.060)	I (0.015)	J (0.465)	K (0.125)	M (0.055)	O (0.280)		
HMS7	6	J (0.153)	K (0.138)	L (0.418)	M (0.051)	N (0.086)	O (0.153)		
ASB17	7	G (0.145)	H (0.015)	M (0.145)	N (0.130)	O (0.057)	Q (0.015)	R (0.489)	
HTG6	4	G (0.388)	J (0.126)	O (0.469)	P (0.015)				
AHT5	5	J (0.277)	K (0.025)	M (0.131)	N (0.545)	O (0.020)			
ASB23	7	I (0.611)	J (0.106)	K (0.106)	L (0.055)	Q (0.111)	S (0.005)	U (0.005)	
HMS6	5	K (0.101)	L (0.323)	M (0.070)	O (0.085)	P (0.419)			
ASB2	6	I (0.175)	K (0.050)	M (0.050)	O (0.085)	Q (0.635)	Q (0.005)		
HTG10	8	I (0.041)	K (0.077)	L (0.597)	M (0.061)	O (0.139)	P (0.005)	R (0.051)	S (0.025)
HTG7	4	K (0.329)	N (0.026)	O (0.638)	Q (0.005)				
HMS3	6	I (0.199)	M (0.091)	N (0.449)	O (0.015)	P (0.234)	Q (0.010)		
HMS2	8	H (0.070)	I (0.030)	K (0.030)	L (0.515)	M (0.140)	P (0.035)	Q (0.015)	R (0.165)

TABLE 3. Observed (HObs), expected heterozygosity (HExp), polymorphic information content (PIC), combined non-exclusion probability first parent (NE-1P) and second parent (NE-2P) of 14 microsatellite markers in Arabian horses

Locus	No. of allele	HObs	HExp	PIC	NE-1P	NE-2P	HWE
VHL20	7	0.770	0.777	0.738	0.618	0.439	NS
HTG4	4	0.570	0.546	0.486	0.849	0.705	NS
AHT4	6	0.760	0.686	0.636	0.727	0.556	NS
HMS7	6	0.776	0.753	0.718	0.639	0.456	NS
ASB17	7	0.729	0.701	0.664	0.700	0.517	NS
HTG6	4	0.636	0.615	0.533	0.809	0.673	NS
AHT5	5	0.677	0.610	0.547	0.803	0.650	NS
ASB23	7	0.657	0.592	0.559	0.798	0.619	NS
HMS6	5	0.697	0.701	0.648	0.716	0.546	NS
ASB2	6	0.590	0.557	0.518	0.828	0.660	NS
HTG10	8	0.619	0.611	0.583	0.775	0.590	NS
HTG7	4	0.511	0.486	0.394	0.883	0.787	NS
HMS3	6	0.418	0.699	0.647	0.722	0.549	***
HMS2	8	0.680	0.649	0.649	0.713	0.529	NS
Mean	5.714	0.663	0.646	0.600	0.9999	0.9999	

TABLE 4. Parentage examination by 14 microsatellite loci in Arabian horses. ES: Sire, ED: Dam, EP: Foal

Marker	VHL20	HTG4	AHT4	HMS7	ASB17	HTG6	AHT5	ASB23	HMS6	ASB2	HTG10	HTG7	HMS3	HMS1
ES	L/L	L/M	J/O	L/O	R/R	G/P	J/N	I/Q	K/L	I/Q	L/L	K/O	I/N	L/R
ED	R/M	K/O	J/J	L/N	G/G	G/G	N/N	I/I	P/P	Q/O	M/O	K/K	N/N	L/L
EP	L/R	K/L	J/O	L/O	G/R	G/P	N/N	I/I	K/P	I/Q	L/O	K/O	N/N	L/L

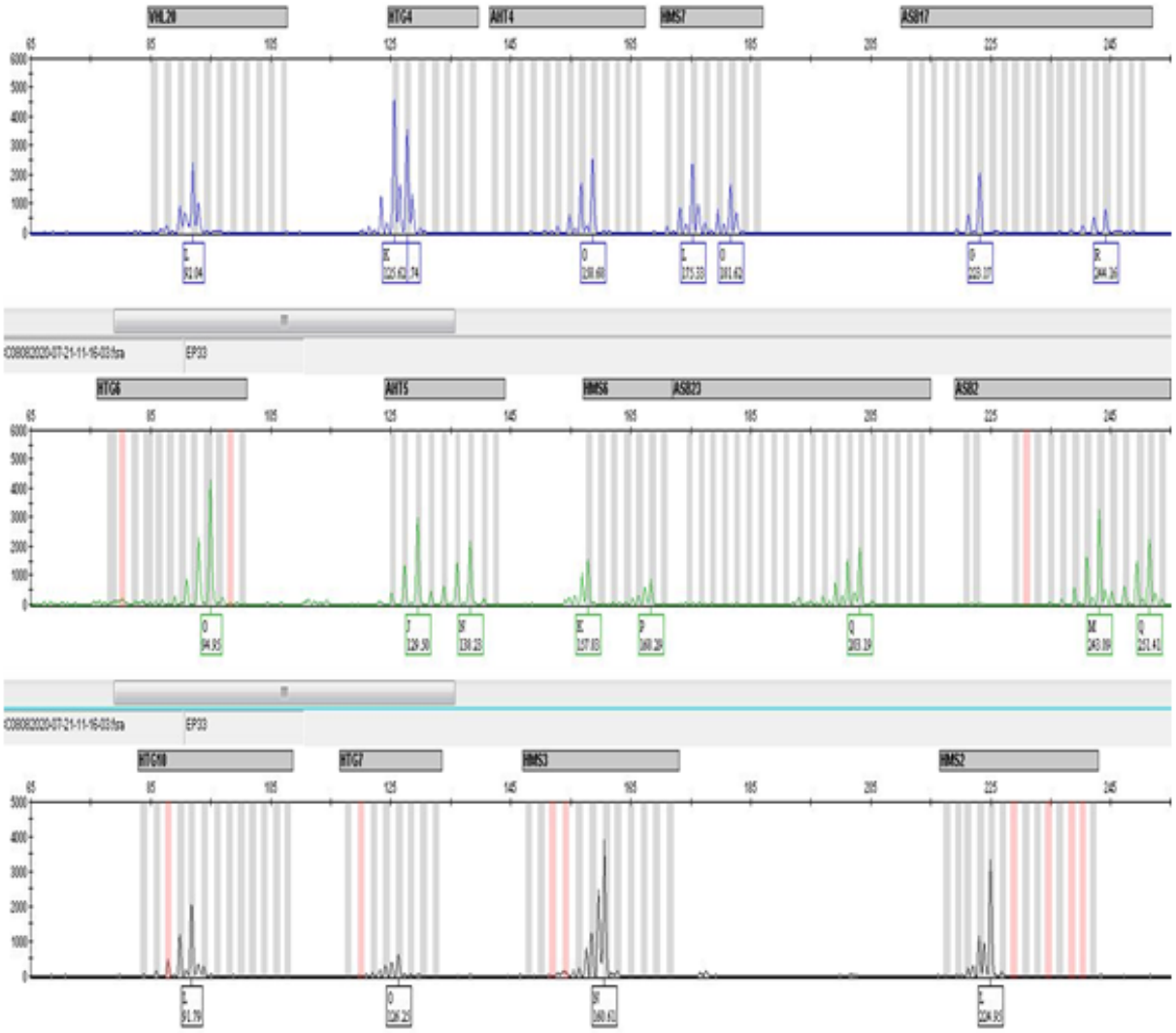

Fig. 1. Electropherogram showing different allele fragment size of 14 microsatellite markers used in the study.

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تحليل المايكروساتليت لتعدد الأشكال الوراثية وتحديد النسب في الحصان العربي

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الملخص

كان الهدف من هذا البحث هو استخدام microsatellite DNA typing لإنشاء طريقة لاختبار النسب للخيول العربية. تم تصميم مجموعة بيانات كبيرة تتكون من 100 عينة، تتكون من 73 مهراً لاختبار النسب و 7 أحصنة إلى جانب 20 فرسًا أنثي لتحديد المحتوي الجيني. تم استخراج الحمض النووي الجيني من عينات الدم وتحليله باستخدام 14 ميكروستاليت ماركر (VHL20 و HTG4 و AHT4 و HMS7 و ASB17 و HTG6 و AHT5 و ASB23 و HMS6 و ASB2 و HTG10 و HTG7 و HMS3 و HMS2). تضمنت طريقة التنميط الجيني تفاعل البلمرة المتسلسل المتعدد ، مما يدل على التضخيم الفعال عبر جميع المواضع المستهدفة. كشف التحليل عن مجموعة من 4 إلى 8 أليلات لكل موضع، بمتوسط قيمتة 6.36 في الخيول العربية. تراوح قيمة ال heterozygosity المتوقع من 0.548 إلى 0.831 (متوسط 0.699)، مع احتمال استبعاد إجمالي قدره 0.9998 عبر 14 موقعًا للميكروستاليت .والجدير بالذكر أن مواقع ASB2 و ASB17 و ASB23 و HMS7 و HTG10 أظهرت قيمًا عالية نسبيًا لمحتوى المعلومات متعددة الأشكال (أكبرمن 0.7). من بين 82 مهراً تم تقييمها، استوفت 78 مهراً معايير التوافق المندلية، مما يؤكد موثوقية طريقة DNA typing للتحقق من النسب والتعرف الفردي على الخيول العربية. تؤكد هذه النتائج على الإمكانات العالية microsatellite DNA typing لتعزيز التحقق من النسب وإدارتها في برامج تربية الخيول العربية.

الكلمات الدالة: المايكروستاليت ، الخيول العربية ، تعدد الأشكال، التحقق من النسب.