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Determining genetic markers for twinning in Egyptian goat breeds

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ABSTRACT

Blood samples were collected to determine genetic markers associated with twinning in the Egyptian Al-Barqi, Al-Baladi and Al-Zaribi goat breeds. Animals were selected from experimental stations of the Animal Production Research Institute using pedigree records. Both Al-Zaraibi and Al-Baladi are among the most productive breeds of Egyptian goats, and each of them has been divided into a prolific and non-prolific group. DNA extraction process from blood samples was handled by two molecular fingerprinting techniques. Random polymorphic DNA (RAPD) molecular markers were used to characterize the three breeds. Micro-satellites, or simple sequence repeats (SSRs), were used to detect genetic polymorphisms within each of the prolific and non-prolific groups. The DNA micro satellites used are: ILST019, INRA0005, MAF0065, SRCRSP0005, SRCRSP0024, McM0527 and OarFCB0020. For constructing a combined Dendrogram dealing with genetic relationships among the three goat breeds studied, the data generated from molecular markers were introduced to SPSS package programme according to binary values (1,0); Bands were scored as 1 if present or 0 if absent. The output results involved both different hierarchical pair-wise distance (UPGMA) and constructed dendrogram .The RAPDPCR analysis indicated that each goat population had unique banding patterns. On the other hand, the RAPD-PCR analysis of amplified DNA samples showed that it can be used to differentiate between prolific and non-prolific breeds of goats. The selected micro satellite markers showed little informational abilities about prolific and non-productive females with the exception of INRA0005 and OarFCB0020. INRA0005 was the most polymorphic and therefore the most informative.

Keywords: *Egyptian goat breeds, genetic markers, litter size.*

INTRODUCTION

Since reproductive performance is an important criterion in animal production, there is great interest in prolific breeds. With the discovery of the **Booroola** gene (or FecB gene) in the 1980s in Australian pippin Merino sheep, research into reproduction (or birth size) escalated. For most traits, such as those for meat and milk production, many genes interact to produce the final effect, and it is difficult to determine which single gene has an important role to play. To accurately identify individuals with superior genes, it is often necessary to spend a great deal of time and effort measuring the performance of large numbers of their relatives (including offspring).The discovery of DNA polymorphisms and the development of molecular biology offer the possibility of identifying genetically superior individuals in a more direct way by identifying functional parts of small pieces of DNA called microsatellites. If a particular microsatellite is near a functionally useful gene (for example, a gene for larger litter size), they are likely to be inherited together. This microsatellite can then be used as a marker for a functional gene. The first stage in using these is to create a marker (or linker) map covering the entire genome (i.e. a genome map).Compared to other native species, goats are considered the ideal animal model for climate change because of their high tolerance to heat and drought, their ability to survive in limited pastures, and their high resistance to disease (**Ahlawat et al 2015 and 2016).**There are many Egyptian goat breeds, including three main breeds: Al-Zaribi, Al-Baladi, and Al-Barqi (Galal et al. 2005) . To date, these strains have not been fully characterized genetically, especially with the use of advanced biotechnology techniques. Therefore, this study

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aims to know the genetic variation at the DNA level between and within these three breeds for the trait of twinning, which is considered one of the most important economic traits. This information may have an important role in programs for genetic improvement of goat breeds in Egypt.

MATERIALS AND METHODS

Experimental animals

One hundred and forty-two adult females of different ages from three Egyptian goat breeds (Barqi, Baladi, and Zarabi) were selected according to litter size using pedigree records from experimental stations belonging to Animal Production Research Institute (APRI) , retaining only individuals with three generations. Both Al-Zaribi and Al-Baladi are among the most prolific breeds of Egyptian goats, and accordingly they were divided into two groups: prolific and non-prolific.

Genetic characterization:

Molecular labeling in the present work was performed using DNA from blood samples according to Sambrook et al. (1989). Blood samples were collected via the jugular vein using 1.5 ml tubes containing the anticoagulant disodium EDTA. All samples were then stored at -20°C until needed. Genomic DNA was extracted from samples using the Ease Pure® Blood Genomic DNA Kit. DNA extraction was processed by two molecular fingerprinting techniques; RAPD markers and SSR markers to describe the three breeds

Random amplified polymorphic DNA (RAPDs):

1-RAPD-PCR was performed to capture molecular fingerprints of populations and estimate phylogenetic relationships. RAPD markers were developed as a technology that uses random parts of the genome. One approach involves PCR amplification of unknown DNA fragments, known as random amplified polymorphic DNA (RAPD) analysis.

2- Conditions for amplification and electrophoresis:

Amplification conditions were performed according to Williams et al. (1990).

SSR markers (i.e., short tandem repeats):

SSR markers have been developed as a technology. It uses random parts of the genome (i.e. short tandem repeats) known as microsatellites. For goats, a list of eighteen microsatellites, 14 of which combined into two multiplexes, were recommended by the ISAG and tested by many laboratories for goat diversity studies. In the present report, initial screening of a total of 7 DNA microsatellites, out of the above-mentioned ones, was undertaken to detect the genetic polymorphisim among and within each one of the two groups of prolific and non-prolific. They are: ILST019, INRA0005, MAF0065, SRCRSP0005, SRCRSP0024, McM0527 and OarFCB0020.

Data analysis:

RAPD-DNA marker data were obtained for 91 adult females of different ages representing the three Egyptian goat breeds, as well as SSR marker data for two equal subgroups, the prolific and the unprolific, were analyzed.

Dendrogram Construction:

To create a common dendrogram that addresses the genetic relationships between the three goat breeds studied, the data generated from the molecular markers were entered into SPSS according to binary values (1,0); Bands were scored as 1 if present or 0 if absent. The output results involved both different hierarchical pair-wise distance (UPGMA) and constructed dendrogram.

RESULTS AND DISCUSSION

(I) Initial screening of ten random RAPD-DNA primers (i.e., DNA markers) was performed with the three goat breeds to detect genetic polymorphisms between and within each of the three populations. Figures 1 through 10, for example, represent results for Zaraibi goat population.

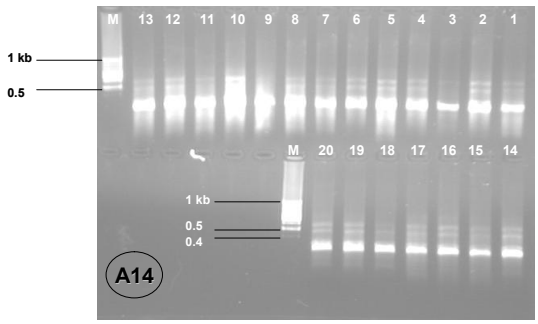

Figure (1): Primer A14.*

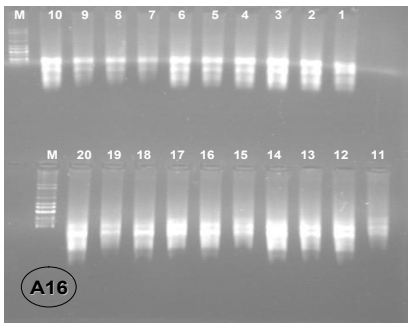

Figure (2): Primer A16.*

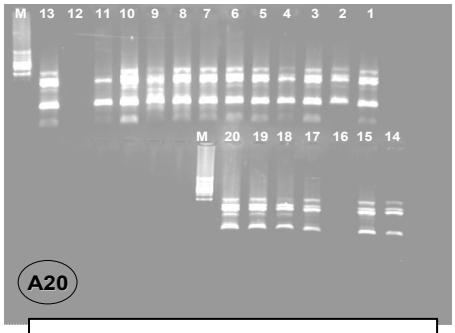

Figure (3): Primer A20.*

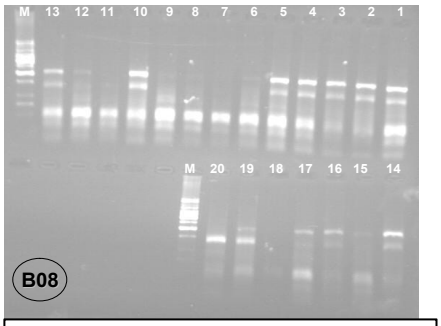

Figure (4): Primer B08.*

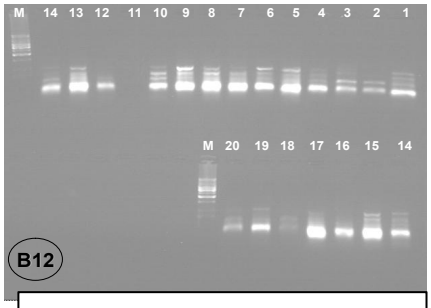

Figure (5): Primer B12.*

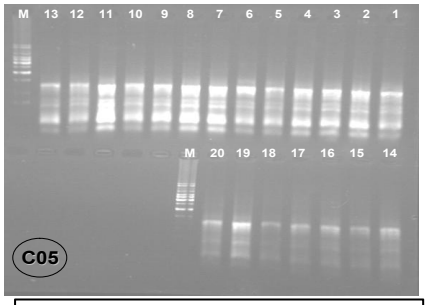

Figure (6): Primer C05.*

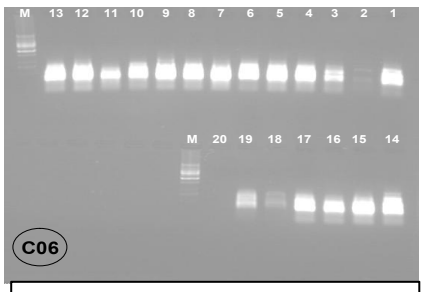

Figure (7): Primer C06.*

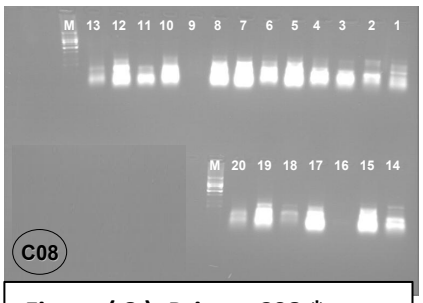

Figure (8): Primer C08.*

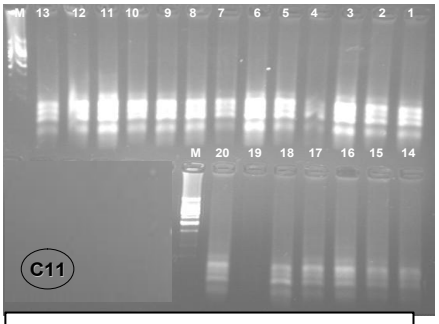

Figure (9): Primer C11.*

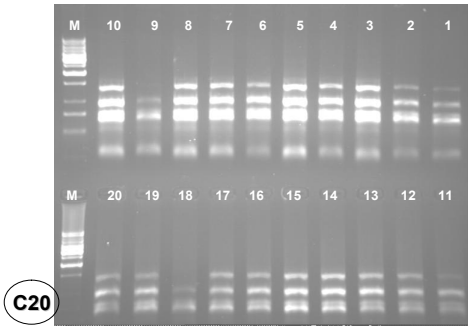

Figure (10): Primer C20.*

** *RAPD fingerprints for individual samples of the Zaraibi breed.*

M = DNA marker; Lanes 1-13 represent prolific females and lanes 14-20 represent non-prolific females.

The dendrogram of this group of goats (Figure 11) showed that female Zaraibi were divided into two main groups, each group containing a mixture of prolific and non-prolific females. Within each group the animals were again divided into two main subgroups: The smallest consists of prolific females from the El-Serw station and non-prolific females from the Sakha station, and the largest consists of prolific females from the Sakha station and non-prolific females from the Sakha station. This distribution reflects the degree of homogeneity in this breed. On the other hand, RAPD-PCR analysis of amplified DNA samples showed that it can be used to distinguish between prolific goat breeds, represented in our study by the prolific Zaraibi and Baladi females, and non-prolific ones. A total of 7 DNA microsatellites were initially screened for genetic polymorphisms between and within each of the two groups of the Zaraibi females (i.e., prolific and non-prolific) using the SSR technique. The selected microsatellite markers showed little information capabilities about prolific and non-prolific females of the Zaraibi breed except INRA0005 and OarFCB0020. INRA0005 was the most polymorphic and thus the most informative. Additional markers are being tested in order to complete genetic characterization and determine phylogenetic relationships.

And so, thus, the results of the SSR technique using microsatellite markers showed that it was able to separate prolific and non-prolific Zaraibi individuals with enough accuracy.

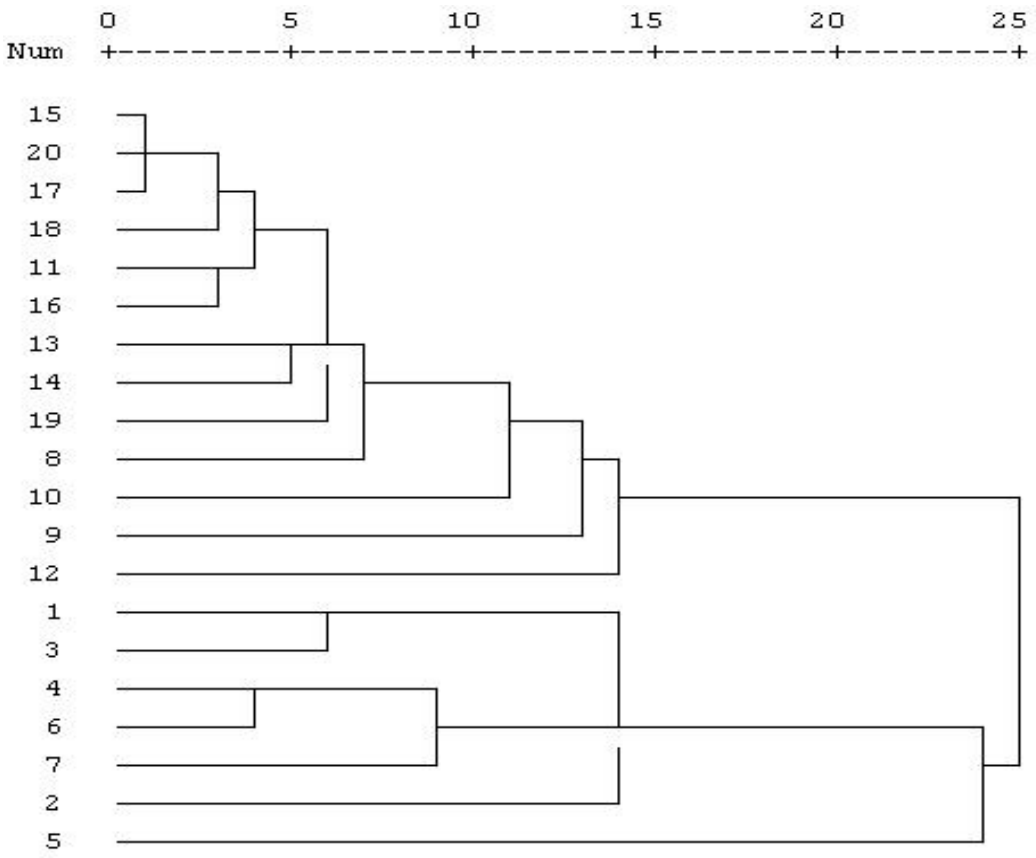

Figure (11): Figure (11): Average genetic correlation among female Zaraibi , taken from Sakha station

(II) In this study, a total of 7 DNA microsatellites (ILST019, INRA0005, MAF0065, SRCRSP0005, SRCRSP0024, McM0527, and OarFCB0020) (Figures 12-18) were initially screened for genetic polymorphisms between and within each one of the two groups of the Zaraibi females (i.e. prolific and non-prolific) using the SSR technique (15 prolific and 15 non-prolific females).

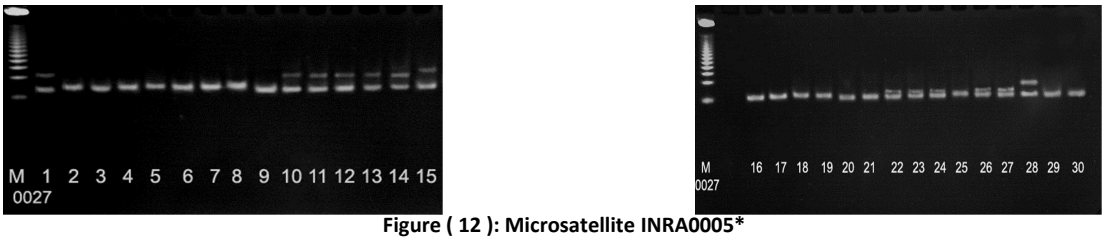

Figure (12): Microsatellite INRA0005*

Figure (13): Microsatellite MAF0065*

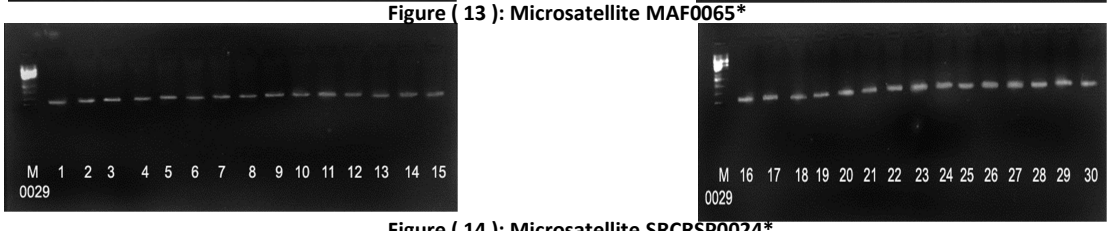

Figure (14): Microsatellite SRCRSP0024*

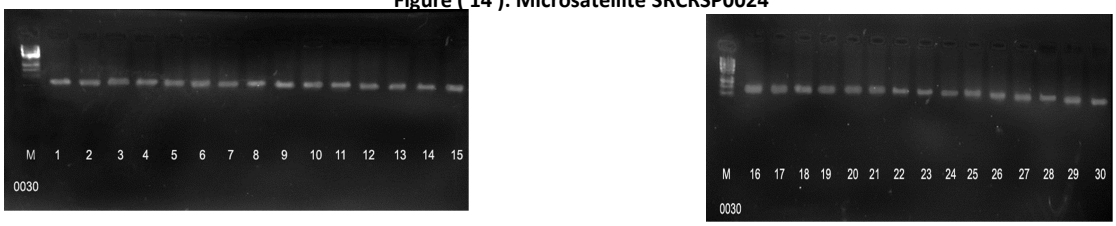

Figure (15): Microsatellite ILST019*

Figure (16): Microsatellite SRCRSP0005*

Figure (17): Microsatellite OarFCB0020*

Figure (18): Microsatellite McM0527*

** SSR fingerprints of individual samples for the Zaraibi breed.*

M = DNA marker; Lanes 1-15 represent prolific females and lanes 16-30 represent non-prolific females.

The dendrogram of this population of goats (Figure 19) showed that Zaraibi females were divided into many groups each one contains a mixture of both prolific and non-prolific females.

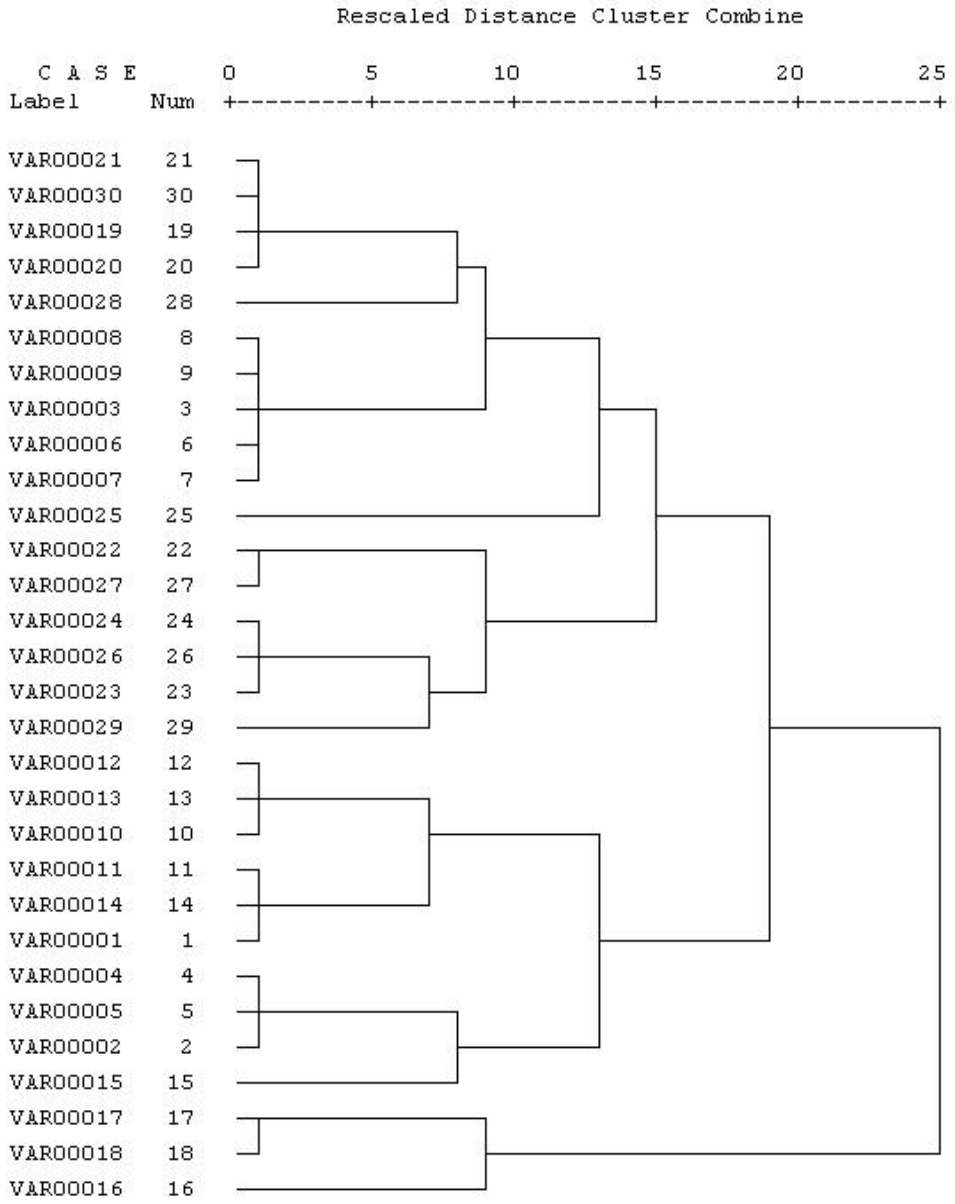

Figure (19): Average genetic linkage among Zaraibi females.

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Among the main Egyptian local goat breeds Zeraibi and Baladi are famous for their high litter size (i.e. twining rate), however, Barki breed came lastly. This variation in such important trait could be shown based upon information at the DNA level by using various molecular genetic techniques such as RAPD, SRAP, AFLP, ISSR, SSR and others which are widely used since 1990s to estimate genetic variation in livestock at molecular level. Molecular marker is so good and very important tool in molecular genetic research for determining the genetic variation and biodiversity with high levels of accuracy and reproducibility. Advances in molecular genetic have created new opportunities for the improvement of litter size in goat. On the other hand, FecB gene is an autosomal dominant gene with a large effect on ovulation rate. Many studies concluded that the effect of FecB was increasing ovulation rate in sheep by about 1.6 (George *et al.*, 2002; Piper and Bindon 1982). Recent discoveries showed that high prolificacy in sheep carrying the Booroola gene (FecB) is the result of a mutation in the *BMPIB (ALK 6)* receptor; (Mulsant *et al.*, 2001). With the use of microsatellite markers (Montgomery *et al.*, 1993), it became possible to predict FecB genotype in males and females prior to sexual maturity. Random amplified polymorphic DNA (RAPD) analysis was used to estimate genetic diversity and relationship in much research work in sheep and goats (Ali, 2003; Rahman *et al.,* 2006).

CONCLUSION

Our study revealed that the RAPD-PCR analysis of the bulked DNA samples showed that it can be used to differentiate between prolific goat breeds, represented in our study by the prolific Zaraibi and Baladi females, and non-prolific ones, represented here by the non-prolific Zaraibi and Barki females. The RAPD-PCR technique was able to separate between prolific and non-prolific Zaraibi females and also between Sakha and El-Serw individuals (i.e. between animals of different origin from the same breed. It was also able to separate among Zaraibi breed and the two other breeds. On the other hand, selected microsatellite markers showed little informative capacities about the prolific and non-prolific females of the Zaraibi breed except INRA0005 and OarFCB0020. The INRA0005 was the most polymorphic, thus the most informative.

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الملخص العربى

تحديد العلامات الوراثية للتوأمة في سلالات الماعز المصرية

حلمي مطاوع 1، رضا عنوس 2، محمد رشيد 3، عبد الحليم هبة 1

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2 كلية الزراعة جامعة عين شمس قسم الإنتاج الحيواني

3 كلية الزراعة جامعة عين شمس قسم الوراثة

تم جمع عينات دم من 142 أنثى بالغة من ثلاث سلالات ماعز مصرية (البرقي، البلدي، والزرايبي) لتحديد العلامات الوراثية المرتبطة بصفات الخصوبة. تم اختيار الحيوانات من المحطات التجريبية لمعهد بحوث الإنتاج الحيواني (APRI)، وفقًا لخاصية حجم الولادة باستخدام سجلات النسب. يعد كل من الزرايبي والبلدي من أكثر سلالات الماعز المصرية إنتاجية ولقد تم تقسيم كل منهم إلى مجموعتين غزيرة الإنتاج وغيرغزيرة . تمت معالجة عملية استخراج الحمض النووي من عينات الدم من خلال تقنيتين للبصمة الجزيئية حيث تم استخدام الواسمات الجزيئية للحمض النووي متعدد الأشكال العشوائي (RAPD) لتوصيف السلالات الثلاثة و تم استخدام الأقمار الصناعية الصغيرة، أو تكرارات التسلسل البسيط (SSRs)، للكشف عن تعدد الأشكال الجينية داخل كل مجموعة من المجموعات الغزيرة وغير الغزيرة . الأقمار الصناعية الدقيقة للحمض النووي المستخدمة هي: ILST019، INRA0005، MAF0065، SRCRSP0005، SRCRSP0024، McM0527 وOarFCB0020. لإنشاء مخطط شجيرات مشترك يتناول العلاقات الوراثية بين سلالات الماعز الثلاثة التي تمت دراستها، تم إدخال البيانات الناتجة من الواسمات الجزيئية إلى برنامج SPSS وفقًا للقيم 1 إذا كانت موجودة أو صفر إذا كانت غائبة. أظهر تحليل RAPD-PCR لعينات الحمض النووي المضخمة أنه يمكن استخدامه للتمييز بين سلالات الماعز الغزيرة وغيرغزيرة الأنتاج. أظهرت علامات الأقمار الصناعية الصغيرة المختارة قدرات معلومات قليلة حول الإناث الغزيرة وغير غزيرة الأنتاج باستثناء INRA0005 وOarFCB0020. كان INRA0005 هو الأكثر تعددًا للأشكال وبالتالي الأكثر إفادة.