Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Association of Microsatellite Markers on Chromosome Seven with Milk Production and Fertility Traits in Egyptian Buffalo

Mohamed Attia Ahmed Awad* and Samy Abou-Bakr

Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.

Abstract

THE aim of this study was to investigate the association of 16 microsatellite markers located on chromosome seven with milk production and fertility traits in Egyptian buffalo. Data were collected from 995 lactation records of 296 multiparous Egyptian buffalo during the period from 2000 to 2022. All the studied microsatellite loci were found to be highly polymorphic. The average estimates for molecular genetic parameters across the 16 microsatellite markers were 8.750, 7.306, 0.857, 0.855, 0.741, 2.033, and -0.071 for observed alleles, effective alleles, observed heterozygosity, expected heterozygosity, polymorphic information content, Shannon's information index, and fixation index, respectively. Association analysis revealed significant associations for 14 out of the 16 microsatellite markers with the productive and reproductive traits under investigation ($P < 0.05$, $P < 0.01$, and $P < 0.001$). Specifically, seven microsatellite markers (BM415, BM143, BMC4203, BMS483, RM28, ILSTS93, and ILSTS97) were significantly associated with milk production traits; three markers (BMS2508, BM4311, and BP7) were significantly related to fertility traits; and four markers (BMS2460, BM1329, BM1236, and AFR227) revealed significant associations with multiple traits, encompassing both milk production and fertility traits. The current findings shed more light on unraveling the genetic background underlying milk production and fertility traits in Egyptian buffalo. The significant associations between the investigated microsatellite markers and various phenotypic traits provide evidence that chromosome seven harbors putative causal genetic variants and key candidate genes affecting these economically important traits in Egyptian buffalo.

Keywords: Association, Egyptian buffalo, fertility, microsatellites, milk traits.

Introduction

The most economically important traits in livestock, such as milk production and fertility, are complex quantitative traits. Environmental conditions and a large number of segregating genomic loci, known as quantitative trait loci (QTL), combine to influence these traits [1]. Advances in molecular genetics techniques have significantly enhanced the ability to identify and map the QTL using DNA markers, which can pinpoint the genes underlying trait variation [2]. QTL mapping is performed by testing the association between genetic variation at marker loci and phenotypic variation in a trait [3]. Identifying QTL is the first step of marker assisted selection (MAS), where significant genetic markers associated with desirable phenotypes are integrated into the genetic evaluation and parent selection processes. This approach substantially accelerates genetic improvement [4, 5].

Microsatellite loci, also known as short tandem repeats (STRs), are genomic regions characterized by the repetition of nucleotide motifs ranging from one to six base pairs, found throughout the genome of prokaryotes and eukaryotes [6]. These loci are considered powerful genetic markers due to their high level of polymorphism, co-dominance inheritance, reproducibility across the genome, selective neutrality, and relative ease for genotyping

*Corresponding author: Mohamed A. A, Awad, Email: mohamed.awad@agr.cu.edu.eg, Tel.: 01003043114
(Received 09 October 2024, accepted 28 November 2024)
DOI: 10.21608/EJVS.2024.327032.2418

and analysis [7]. Based on these characteristics, microsatellite markers have been widely used for a broad range of genetic studies, including analysis of genetic diversity [8], population structure and admixture [9], phylogenetic relationships [10], and assessments of parentage and pedigree accuracy [11]. Additionally, microsatellite markers have been extensively used in mapping important QTL in cattle [12], buffalo [13], sheep [14], goats [15], and poultry [16].

Despite the widespread use of SNP arrays for genome-wide coverage in genomic prediction, microsatellite markers still hold value in specific contexts. Microsatellites are still cost-effective and accessible tools, particularly in resource-limited settings where the adoption of high-density SNP arrays might not be feasible. Furthermore, the existing microsatellite data accumulated over decades allows for continuity in ongoing research and breeding programs. Additionally, in buffalo, where SNP arrays might not yet offer as comprehensive coverage as in cattle, microsatellites can still play a crucial role in identifying key QTLs.

Bovine chromosome six (BTA6) is one of the most significant chromosomes in QTL mapping for various quantitative traits, including milk yield and its composition [17, 12, 5], fertility [18], diseases resistance and survival [19, 20], as well as growth and carcass traits [21]. Comparative cytogenetic maps show extensive similarity in chromosomal banding patterns between cattle and river buffalo karyotypes [22, 23]. Notably, chromosome seven in buffalo (*Bubalus bubalis* 7, BBU7) has conserved synteny with bovine chromosome six. This suggests that BBU7 may harbor the same genetic content, including QTL or microsatellite markers, as BTA6. Consequently, bovine microsatellites may be applicable for use in buffalo [22]. Therefore, the objective of this study was to examine the association of 16 microsatellite markers on BBU7 with productive and reproductive traits in Egyptian buffalo.

Material and Methods

Buffalo and phenotypic data

Phenotypic data were collected from 995 lactations of 296 multiparous Egyptian buffalo over the period from 2000 to 2022. These buffalo were genetically unrelated and housed in six herds distributed across different geographical regions. The relatedness between the sampled animals was checked through the information provided by the herd's owner and the available pedigree records.

Five production traits and four fertility traits were examined from records of productive and reproductive performance. The milk production traits included total milk yield (TMY, kg); 305-day milk yield (305-dMY, kg, calculated according to Khan and Chaudhry [24] as: total milk yield*305/lactation length); lactation length (LL, days); daily milk yield (DMY, kg), and dry period length (DPL, days). The fertility traits included the number of services per conception (NSPC); post-partum first service (PPFS, days); days open (DO, days), and calving interval (CI, days). Descriptive statistics for the milk production and fertility traits of the Egyptian buffalo in the study are presented in Table 1.

Blood sampling and DNA extraction

Approximately 10 millilitres of blood were collected from the jugular vein of each buffalo (n=296) and stored in sterile Falconer tubes containing 0.5 ml Na2EDTA (0.5 M) as an anticoagulant material. Blood samples were stored at –20°C until DNA extraction. Genomic DNA was extracted from white blood cells using Fermentas® kits (cat.no.k0512, Fermentas Life Science/Thermo Fisher Scientific, EU), according to the manufacturer's guidelines described in the kit protocol. The integrity, concentration, and purity measurements of the genomic DNA samples were assessed by running the samples on 0.8% agarose gel and using ScanDrop® 200 (Anyltikajena, UK). All the stock DNA samples were prepared at a concentration of 50 ng/ µl and stored at –20°C till further use.

Selection of microsatellite markers

Previous studies that suggested their associations with quantitative traits in cattle and buffalo led to the selection of a total of 16 adjacent microsatellite markers on chromosome seven of the buffalo genome. Information on the considered microsatellite markers in the present study is presented in Table 2.

Polymerase chain reaction amplification and genotyping

Polymerase chain reaction (PCR) amplification was conducted by a thermal cycler PCR machine (G-Storm®, Gene Technologies, UK). The optimal PCR amplification mixture had a final volume of 20 µl and included 50 ng/µl of genomic DNA, 5 Pmol of each forward and reverse primer (Bioneer, Daejeon, South Korea), 200 µM dNTP mix, 1U Taq DNA polymerase (FIREPol®, Solis BioDyne, Estonia), 1.5 mM MgCl2, and 10× PCR reaction buffer (pH 8.8). The PCR cycling profile consisted of the primary denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at varying temperatures specified for each primer (Table 2) for 60 seconds, and extension at 72°C for 45 seconds. A final extension was conducted at 72°C for 5 minutes, followed by storage at 4°C.

To validate the success of the PCR amplification, each primer's PCR product was subjected to 2% agarose gel stained with ethidium bromide (Sigma Aldrich, USA), using the horizontal electrophoresis (Biometra® EU) and visualized under UV light. For the genotyping assay, successful PCR-amplified samples were separated on a 12% polyacrylamide gel using a vertical electrophoresis system (Biometra® EU) under denaturing conditions. A 100 bp DNA ladder (Promega®, USA) was loaded on a separate well as a molecular weight standard to measure the actual allele sizes of the PCR products. After the vertical electrophoresis step, the gel was stained with ethidium bromide and imaged under UV light using the gel documentation system (Gel DocXR®, Bio-Rad®, USA). Genotypes were determined by measuring the sizes of bands for each sample compared to the ladder using Quantity One® 1-D analysis software.

Statistical analysis

Microsatellite polymorphisms analysis

The raw genotyping data were processed and converted to the appropriate format using CONVERT version 1.31 [27] to prepare the input files for further genetic analysis. The converted data were analyzed using POPGENE 1.32 software [28] to calculate the observed number of alleles per locus (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Shannon's information index (I) and fixation index (inbreeding coefficient) per locus (F). The polymorphic information content (PIC) for each marker was calculated using CERVUS version 3.0.3 software [29]. The deviation from Hardy–Weinberg equilibrium (HWE) was assessed using a Chi square test performed with POPGENE 1.32 software.

Association analysis

Associations between the phenotypes of the studied traits and the genotypes of the microsatellite markers were evaluated using univariate analysis of variance within the general linear model (GLM) framework, utilizing IBM SPSS Statistics software (Version 22, Armonk, NY, USA). Associations were considered statistically significant at a P-value < 0.05. The linear model used for the analysis was as follows:

$$Y_{ijklmn} = \mu + H_i + S_j + Y_k + P_l + G_m + e_{ijklmn}$$

Where, Y_{ijklmn} is the phenotypic value of milk production and fertility traits of n^{th} individual; μ is the overall mean; H_i indicates the fixed effect of i^{th} herd (six herds); S_j signifies the fixed effect of j^{th} season of calving (j:1, 2, 3 and 4), where, 1: winter (December to February), 2: spring (March to May), 3: summer (June to August), and 4: autumn (September to November); Y_k refers to the fixed effect of k^{th} year of calving (2000 to 2022); P_l is the fixed effect of l^{th} parity (l = 1-16); G_m is the fixed effect of m^{th} genotypes of each microsatellite, and e_{ijklmn} is the random error, assumed to be normally independently distributed with mean zero and constant variance, NID (0, $\sigma^2 e$).

Results and Discussion

Microsatellite polymorphisms

Allele size (bp), observed number of alleles per locus (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), polymorphic information content (PIC), Shannon's information index (I), inbreeding coefficient (F), and Hardy–Weinberg equilibrium (HWE) for each microsatellite marker, along with the overall means across all genotyped individuals, are presented in Table 3.

According to the estimates of all molecular genetic parameters (Na, Ne, Ho, He, PIC, I, and F), the microsatellite loci analyzed in the current study demonstrated a high degree of allelic polymorphism. A total of 140 alleles, with an overall mean of 8.750±2.352 alleles per locus, were observed across 16 microsatellite markers. The locus AFR227 exhibited the highest number of observed alleles (13), while loci BM2320 and BM4311 showed the lowest number of alleles (five). The effective number of alleles (Ne) was lower than the observed number, ranging from 4.048 (BM4311) to 12.120 (AFR227), with an overall mean of 7.306±2.290 alleles per locus. The higher observed number of alleles compared to the effective number indicates significant differences in allele frequencies at each locus, suggesting a high proportion of low frequency alleles across the investigated loci [30].

The current estimates of Na are lower than those reported by Abou-Bakr et al. [31], who observed 198 alleles with a mean of 22 alleles per locus using 11 microsatellite markers in Egyptian buffalo. Also, Ünal et al. [32] detected 190 alleles in 837 Turkish water buffaloes genotyped by 20 microsatellite markers. However, Rushdi et al. [13] reported a total of 33 alleles with an average of 4.125 per locus in Egyptian buffalo based on eight microsatellite markers. The mean Ne in our study is notably lower than the 16.502 reported by Abou-Bakr et al. [31] in Egyptian buffalo, but higher than the values of 4.82 and 3.64 reported by Khade et al. [33] and Singh et al. [34] in Pandharpuri and Gojri buffalo, respectively. These differences in Na and Ne across studies may be attributed to variations in the differences in the set of microsatellite marker sets

utilized, their number, and the number of genotyped individuals.

The observed heterozygosity (Ho) values varied from 0.523 (BP7) to 1.000 (BM415, BM1329, and BM1236), with an overall mean of 0.857±0.141 per microsatellite, while the estimated values of expected heterozygosity (He) ranged from 0.768 (BM4311) to 0.919 (AFR227), with a mean of 0.855±0.045 per locus. Heterozygosity is a reliable genetic parameter used to evaluate genetic variation and, consequently, the circumstance of inbreeding level within a breed [35]. In this study, the overall means of both Ho and He were almost equal and greater than 0.5, which reflected the existence of substantial genetic variability among the genotyped individuals and a highly polymorphic state across all studied markers. The higher heterozygosity observed suggests minimal genetic improvement in Egyptian buffalo, random mating, and potential population admixture. Similar high overall means of Ho and He have been reported in Egyptian buffalo [31], Iraqi buffalo [36], Iranian buffalo [37], and Gojri buffalo [34]. However, the Ho and He values in this study are remarkably higher than those reported for Cuban water buffalo [38], Brazilian Murrah and Jaffarabadi buffalo [39], Pakistan buffalo [40], Egyptian buffalo [13], Turkish water buffalo [32] and Indian buffalo [41]. These higher estimates of heterozygosity compared with other studies are expected due to the large sample size and the broader distribution of the genotyped individuals across six different herds in the current study.

The PIC for each locus ranged from 0.618 (BM2320) to 0.859 (BP7), with a mean PIC value of 0.741. PIC is a molecular genetic parameter indicating the informativeness of a microsatellite marker, with values ranging from 0 to 1. According to Botstein et al. [42], microsatellite markers with PIC estimate greater than 0.5 are considered highly informative for diversity and association genetic studies. Consequently, all microsatellite markers used in the present study are highly informative (PIC > 0.5). The overall mean of PIC in this study aligns with those reported by El-Kholy et al. [43], Darestani et al. [37], Khade et al. [33], and Gahlyan et al. [41] in Egyptian, Iranian, Pandharpuri, and Indian buffalo, respectively. On the other hand, the mean PIC value in this investigation is higher than those reported for Pakistani buffalo [40], Cuban water buffalo [44], Romanian buffalo [45], and Turkish water buffalo [32]. However, it is lower than the estimates reported by Abou-Bakr et al. [31] and Merdan et al. [46] in Egyptian buffalo.

Shannon's information index (I), which reflects the degree of allelic polymorphism at each microsatellite locus, indicated that AFR227 was the most polymorphic locus (I=2.528), while BM4311 was the least polymorphic locus (I=1.498) with an overall mean of 2.033±0.305 across all microsatellite markers. The average of (I) estimate in this study is considerably higher than those reported in other buffalo breeds [47, 32].

The fixation index per locus (F) fluctuated from -0.165 (BMS483) to 0.364 (BP7), with a negative overall mean (-0.071). The fixation index assesses the degree of the genetic relationship among individuals and reflects the level of inbreeding as well as the deficiency of heterozygotes within the population. In general, the negative mean estimate of (F) suggests an absence of inbreeding among the individuals studied, which is consistent with the high heterozygosity observed across the microsatellites. Similar negative (F) values have been reported in Murrah [39], Iranian [37], and Romanian [45] buffalo populations. Conversely, a positive inbreeding coefficient has been observed in Cuban water buffalo [44] and Turkish water buffalo [32].

All microsatellite markers, except BMS2508 and BM2320, significantly deviated from the HWE ($P < 0.05$ and $P < 0.001$). This deviation may be attributed to population admixture, resulting in a high rate of gene flow among the studied herds. This is evidenced by the excess of heterozygotes over homozygotes and the negative mean of inbreeding coefficient [47, 48]. Similar deviations from HWE at microsatellite loci have been reported in various buffalo breeds [13, 37, 32].

Association analysis of microsatellite markers with different phenotypic traits

The microsatellite markers that showed significant associations with milk production and fertility traits are presented in Tables 4 and 5. All the studied microsatellite markers revealed significant associations with at least one phenotypic trait except the markers BM4322 and BM2320 which did not reveal any significant effect on the studied trait. With regard to milk production traits, 11 out of the 16 analyzed microsatellite markers exhibited significant associations with various productive traits ($P < 0.05$, $P < 0.01$, and $P < 0.001$).

Markers BM143, BMC4203, and AFR227 were significantly associated with all milk production traits. In dairy cattle, BM143 is a key locus in QTL fine mapping for milk production traits. For example, Ron et al. [49] and Olsen et al. [50] identified significant QTL for milk fat and protein percentages located at 4 cM and 420 kb, respectively, from BM143 in Israeli Holstein and Norwegian dairy cattle. Consistent with the present findings, Mekkawy et al. [51], Rushdi et al. [13] and Rushdi [52] reported that BM143 is significantly associated

with a QTL influencing milk yield in Egyptian buffalo. However, Li et al. [12] found no significant correlation between BM143 and daily milk yield in Xinjiang Holstein cows, and Vani et al. [53] also demonstrated no significant association between BM143 and TMY, 305-dMY, LL, and DPL in Indian water buffaloes.

Ashwell et al. [54] supported the current findings by demonstrating that BMC4203 is significantly linked with QTL affecting udder traits in US Holstein cattle. Similarly, Ashwell et al. [26] found that AFR227 is linked to a putative QTL affecting milk quality in US Holstein cattle. However, Silva et al. [55] reported a non-significant relationship between AFR227 and milk production traits in Gyr cows.

BMS2460 and BMS483 were significantly associated with four productive traits (TMY, 305-dMY, LL, and DMY). These results align with those of Chen et al. [25], who fine-mapped QTL located near the locus BMS2460 that affect milk yield traits in Chinese Holstein cows. Also, Chen et al. [56] identified QTL related to milk constituents at 51 cM near BMS2460 in a Chinese Holstein population. Additionally, Mei et al. [17] found QTL influencing milk yield and its contents within a 0.6 cM interval near BMS483 in Chinese Holstein cows.

Markers BM1329 and RM28 demonstrated significant associations with three milk production traits: BM1329 with TMY, 305-dMY, and DMY; and RM28 with 305-dMY, LL, and DMY. Consistent with these results, Mekkawy et al. [51] reported a significant influence of BM1329 on milk yield in Egyptian buffalo. Chen et al. [25] also fine-mapped QTL located close to BM1329 and RM28 affecting milk yield traits in Chinese Holstein cows. Conversely, Vani et al. [53] did not detect any significant associations between BM1329 and milk performance traits in Indian buffaloes.

The markers ILSTS93 and ILSTS97 showed significant influences on two productive traits (305-dMY and DMY). Our results correspond to those of Attar et al. [57], who proposed the ILSTS93 locus as a potential indicator for QTL influencing milk quality in Iranian Holstein cattle. Furthermore, Ashwell et al. [54] identified a significant link between ILSTS093 and QTL affecting udder traits using the granddaughter design in US Holstein cattle. Moreover, these results are consistent with Mekkawy et al. [51] who revealed that the marker ILSTS97 had a significant influence on milk yield trait in Egyptian buffalo.

The loci BM1236 and BM415 were significantly associated with only one trait each: BM1236 with TMY and BM415 with DPL. Previous studies have suggested associations of BM1236 with milk quality and conformation traits in US and Chinese Holstein cows, respectively [58, 25]. The results regarding BM415 are in line with Li et al. [12], who reported a non-significant association between BM415 and DMY in the Xinjiang Holstein population. Likewise, Vani et al. [53] found no significant relationship between BM415 and TMY, 305-dMY, or LL in Indian water buffalo. However, contrary to our results, they did not find an association between BM415 and DPL. On the other hand, Rushdi et al. [13] demonstrated that the BM415 locus had a significant effect on DMY and protein yield in Egyptian buffalo.

In the current study, the markers BMS2508, BM4311, and BP7 were exclusively associated with fertility traits and did not show any significant linkage with the milk traits under study. These results are comparable to those of Li et al. [12], who revealed that BP7 had a non-significant correlation with DMY in Xinjiang Holstein cows. However, our results contradict the previous linkage studies in dairy cattle, where the markers BMS2508, BM4311, and BP7 exhibited positive significant relationships with QTL for milk composition traits [26, 59, 25]. Also, Chen et al. [56] mapped QTL affecting milk yield traits approximately 55 cM close to the BP7 locus in Chinese Holstein cows. Similarly, Silva et al. [55] localized QTL underlying milk yield traits close to BMS2508 using a daughter design in Brazilian dairy Gyr cows.

Regarding fertility traits, seven microsatellite markers were significantly associated with reproductive performance traits ($P < 0.05$, $P < 0.01$, $P < 0.001$). The markers BMS2508, BM1236, and BP7 revealed significant associations with the NSPC. The PPFS trait was significantly associated with two markers, BMS2460 and BM1329. Additionally, the DO trait was significantly linked to two markers, BMS2508 and BM4311.The CI was significantly influenced by only one locus, AFR227. In contrast to these findings, Thomasen et al. [60] reported no associations between the microsatellites BM1329 and BM4311 with fertility traits in Danish Holstein cows. However, the current results align with those of Wang et al. [61], who found that the BM1329 locus significantly affected reproductive efficiency in Chinese goats.

Interestingly, the microsatellite markers BM1236, BMS2460, BM1329, and AFR227 were identified as genetic markers significantly associated with multiple traits, encompassing both fertility and milk production traits. This pattern may be attributed to pleiotropic effects or closely linked QTL, where each QTL affects different traits.

Our results are supported by genome-wide association studies (GWAS) conducted in Egyptian buffalo using the Axiom® Buffalo Genotyping Array 90 K (Affymetrix). These studies identified several significant SNPs and putative candidate genes on BBU7, which potentially influence milk production and fertility traits in Egyptian buffalo. El-Halawany et al. [62], Abdel-Shafy et al. [63], and Awad et al. [64] identified four SNPs on BBU7 significantly associated with various milk traits. Among these, candidate genes such as CAMK2D, ANK2, TECRL, ADGRL3, and CFAP299 were highlighted for their potential effects on milk production. Additionally, EL Nagar et al. [65] specified two candidate genes, KCTD8 and YIPF, on BBU7 that are putatively involved in fertility traits in the Egyptian buffalo. However, it is important to note that these studies were based on relatively small sample sizes of genotyped animals and limited phenotypic records, which may have reduced the power and precision of candidate genes detection. Nevertheless, these findings align with numerous genomic studies that have demonstrated the presence of candidate genes on BBU7 with potential effects on milk production and reproduction traits in Murrah and Italian buffalo. Notable candidate genes identified in these studies include TCERG1, SH3BP5L, ZNF672, LOC101904981, SLCO6A1, QRFPR, and PPARGC1A [66, 67, 68, 69].

Conclusion

This study provides valuable insights into the genetic architecture influencing milk production and fertility traits in the Egyptian buffalo. The analysis revealed significant associations between several microsatellite markers and economically important traits, suggesting that these markers could serve as useful tools for molecular breeding programs aimed at enhancing productivity and reproductive efficiency in this species. Notably, the findings highlight the potential of chromosome seven as a critical region harboring candidate genes linked to these traits. However, given the limitations of microsatellite markers, further studies employing more comprehensive genomic approaches, such as GWAS and next-generation sequencing (NGS), are essential to refine these associations and pinpoint the causal variants responsible for the observed phenotypic variations. Such efforts would not only advance our understanding of the genetic determinants of key traits in the Egyptian buffalo but also contribute to the development of more effective strategies for their genetic improvement.

Statements & Declarations

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All procedures of the present study have been approved by the Institutional Animal Care and Use Committee (IACUC) at Cairo University, Egypt (approval number: CU-II- F-11-24).

Author Contributions

Mohamed Attia Ahmed Awad: designed the experiment, collected the data and samples, conducted the laboratory procedures and the statistical analysis of data, interpreted the results, and wrote the first version of the manuscript. Samy Abou-Bakr: contributed to reagents and materials preparation and reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

TABLE 1. Descriptive statistics for milk production and fertility traits in the Egyptian buffalo

Traits	N records	Mean	SD	Min.	Max.	CV (%)
Milk production traits						
Total milk yield (TMY, kg)	995	2076	585.46	567	3200	28.21
305-day milk yield (305-dMY, kg)	995	2325	515.41	507	3854	22.17
Lactation length (LL, days)	995	273	54.15	170	397	19.85
Daily milk yield (DMY, kg)	995	7.6	1.69	1.7	12.6	22.17
Dry period length (DPL, days)	604	129	51.53	52	276	39.97
Fertility traits						
Number of services per conception (NSPC)	952	1.4	0.83	1	5	58.93
Post-partum first service (PPFS, days)	948	72	40.39	30	197	56.01
Days open (DO, days)	948	91	50.59	45	294	55.80
Calving interval (CI, days)	649	399	51.84	350	569	12.99

N: number, SD: standard deviation, Min: minimum, Max: maximum, CV: coefficient of variation

TABLE 2. Information on the selected microsatellite markers on chromosome seven in the Egyptian buffalo

Microsatellite markers	Gene bank accession numbers/ reference	Physical map name	Primer sequence (5\-3\)	Annealing Temperature °C
BM4322	G29053	D6S43	F:TATCACCACCATGGCAAATG R:GACTCACCATACTCCAGAGCC	58
BMS2508	G18959	D6S44	F:TTTCTGGGATTACAAAATGCTC R:TTTCTTAGGGGAGTGTTGATTC	56
BMS2460	G19012	D6S45	F:TGGACTGTCAAACGTAGGGT R:CAAGTCAGGAAAGATGAAACTTG	58
BM415	G18413	D6S10	F:GCTACAGCCCTTCTGGTTTG R:GAGCTAATCACCAACAGCAAG	54
BM143	G18387	D6S13	F:ACCTGGGAAGCCTCCATATC R:CTGCAGGCAGATTCTTTATCG	58.5
BM2320	G18497	D6S6	F:GGTTCCCAGCAGCAGTAGAG R:CCCATGTCTCCCGTTACTTC	58
BMC4203	G19110	D6S20	F:GCAAATGTAAGCTGAAGGCC R:CCTGGGAAATCCCATGGAC	60
BM1329	G18422	D6S14	F:TTGTTTAGGCAAGTCCAAAGTC R:AACACCGCAGCTTCATCC	58.7
BM1236	G18382	D6S9	F:CTGAACCACCACGGAAGC R:AGTCCATGGGGTTGCAAG	54
BMS483	G18848	D6S51	F:GGTATGAGACCAGGTGTGGG R:CAGGGCCACATTTCCAAG	58.5
BM4311	G18522	D6S8	F:TCCACTTCTTCCCTCATCTCC R:GAAGTATATGTGTGCCTGGCC	58
BP7	Chen et al. [25]	D6S7	F:GACCTTTTCACTGCCCTCTG R:TTTATTTCTGAGTGTTTGGGGC	56
RM28	Chen et al. [25]	D6S4	F:CTACAGTCATGGGTCTGAAAGAG R:ATCTTCAGCCTGGCCTGAGAG	62
AFR227	Ashwell et al. [26]	D6S18	F:GACCAACTGAGTGCATGCACG R:TCATTGAGCAGGAGTAGGATTGAGA	58.5
ILSTS93	L37240	D6S22	F:TGAAATATACCTGAGTAGCAGC R:TTGTTTTAACTCCCCACCCC	58.7
ILSTS97	L37241	D6S23	F:AAGAATTCCCGCTCAAGAGC R:GTCATTTCACCTCTACCTGG	58

F: Forward primer; R: Reverse primer.

TABLE 3. Molecular genetic parameters for microsatellite markers analyzed

Microsatellite markers	Allele size range (bp)	Na	Ne	Ho	He	PIC	I	F	HWE
BM4322	163-187	7	5.491	0.710	0.831	0.679	1.814	0.132	*
BMS2508	89-101	6	4.972	0.733	0.812	0.828	1.694	0.082	NS
BMS2460	69-83	8	7.679	0.912	0.871	0.798	2.058	-0.068	***
BM415	135-153	9	7.867	1.000	0.874	0.760	2.133	-0.158	***
BM143	106-124	10	8.097	0.922	0.878	0.807	2.201	-0.065	***
BM2320	150-158	5	4.557	0.700	0.794	0.618	1.557	0.103	NS
BMC4203	154-180	10	7.751	0.993	0.873	0.704	2.175	-0.157	***
BM1329	129-157	12	10.498	1.000	0.906	0.752	2.420	-0.117	***
BM1236	88-104	9	8.416	1.000	0.883	0.745	2.162	-0.148	***
BMS483	113-133	10	7.257	0.993	0.864	0.702	2.153	-0.165	***
BM4311	95-135	5	4.048	0.808	0.768	0.628	1.498	-0.073	***
BP7	279-329	8	5.628	0.523	0.832	0.859	1.893	0.364	***
RM28	100-120	11	9.658	0.864	0.898	0.692	2.333	0.024	***
AFR227	98-124	13	12.120	0.925	0.919	0.843	2.528	-0.021	***
ILSTS93	197-219	7	4.697	0.875	0.790	0.668	1.717	-0.153	***
ILSTS97	226-262	10	8.166	0.751	0.879	0.771	2.198	0.117	***
Mean		8.750	7.306	0.857	0.855	0.741	2.033	-0.071	
SD		2.352	2.290	0.141	0.045		0.305		

bp: Base pairs, Na: Observed number of alleles per locus, Ne: Effective number of alleles, Ho: Observed heterozygosity, He: Expected heterozygosity, PIC: Polymorphic information content, I: Shannon's information index, F: Inbreeding coefficient, HWE: Hardy–Weinberg equilibrium, NS: not significant, * P<0.05, *** P<0.001, SD: standard deviation.

TABLE 4. Significant microsatellite markers associated with milk production traits in Egyptian buffalo

Trait	Microsatellite markers	F-value	P-value
TMY	**BMS2460**	2.556	0.000***
	BM143	2.785	0.000***
	BMC4203	1.963	0.002**
	BM1329	2.791	0.000***
	BM1236	1.783	0.017*
	BMS483	2.597	0.000***
	AFR227	1.938	0.000***
305-dMY	**BMS2460**	2.340	0.0000***
	BM143	2.100	0.0000***
	BMC4203	1.963	0.003**
	BM1329	2.300	0.0000***
	BMS483	1.780	0.014*
	RM28	1.500	0.042*
	AFR227	2.120	0.0000***
	ILSTS93	1.740	0.033*
	ILSTS97	1.710	0.005**
LL	**BMS2460**	1.581	0.015*
	BM143	1.634	0.010*
	BMC4203	1.529	0.042*
	BMS483	1.657	0.027*
	RM28	1.760	0.007**
	AFR227	1.623	0.002**
DMY	**BMS2460**	2.339	0.000***
	BM143	2.995	0.000***
	BMC4203	1.964	0.002**
	BM1329	2.302	0.000***
	BMS483	1.777	0.014*
	RM28	1.496	0.041*
	AFR227	2.120	0.000***
	ILSTS93	1.743	0.031*
	ILSTS97	1.714	0.004**
DPL	**BM415**	2.070	0.010**
	BM143	1.543	0.025*
	BMC4203	1.795	0.012*
	AFR227	1.730	0.001**

TMY: total milk yield, 305-dMY: 305-day milk yield, LL: lactation length, DMY: daily milk yield, DPL: dry period length, *P < 0.05, **P < 0.01, ***P < 0.001.

TABLE 5. Significant microsatellite markers associated with fertility traits in Egyptian buffalo

Trait	Microsatellite markers	F-value	P-value
NSPC	**BMS2508**	3.090	0.000***
	BM1236	1.654	0.033*
	BP7	1.657	0.038*
PPFS	**BMS2460**	1.682	0.007**
	BM1329	1.555	0.025*
DO	**BMS2508**	1.734	0.044*
	BM4311	2.987	0.007**
CI	**AFR227**	1.410	0.028*

NSPC: number of services per conception, PPFS: post-partum first service, DO: days open, CI: calving interval, *P < 0.05, **P < 0.01, ***P < 0.001

References

1. Getaneh, M. and Alemayehu, K. Candidate genes associated with economically important traits in dairy goats. *Cogent Food & Agriculture*, **8** (1), 2149131, (2022). DOI: 10.1080/23311932.2022.2149131
2. de Lima, L. G., de Souza, N. O. B., Rios, R. R., de Melo, B. A., dos Santos, L. T. A., Silva, K. d., Murphy, T. W. and Fraga, A. B. Advances in molecular genetic techniques applied to selection for litter size in goats (Capra hircus): a review. *Journal of Applied Animal Research*, **48** (1), 38-44 (2020). DOI: 10.1080/09712119.2020.1717497
3. Hu, X., Gao, Y., Feng, C., Liu, Q., Wang, X., Du, Z., Wang, Q. and Li, N. Advanced technologies for genomic analysis in farm animals and its application for QTL mapping. *Genetica*, **136**, 371-386 (2009). DOI 10.1007/s10709-008-9338-7

4. Iheshiulor, O.O.M., Woolliams, J. A., Yu, X., Wellmann, R. and Meuwissen, T. H. E. Within- and across-breed genomic prediction using whole-genome sequence and single nucleotide polymorphism panels. *Genetics Selection Evolution*, **48**, 15-29 (2016). DOI: 10.1186/s12711-016-0193-1

5. Worku, D., Gowane, G. and Verma, A. Genetic variation in promoter region of the bovine LAP3 gene associated with estimated breeding values of milk production traits and clinical mastitis in dairy cattle. *PLoS ONE*, **18** (5), e0277156 (2023). DOI: 10.1371/journal.pone.0277156

6. Susilorini, T. E., Wulandari, D., Furqon, A. Septian, W. A., Saputra, F. and Suyadi, S. Genetic diversity of various goats in East Java based on DNA microsatellite markers. *Tropical Animal Science Journal*, **45** (3), 247-254 (2022). DOI: https://doi.org/10.5398/tasj.2022.45.3.247

7. Dako, S., Laya, N. K., Gubali, S. I., Ardiantoro, A., Nurgiartiningsih, V. M. A., Ciptadi, G., Wulandari, D. and Suyadi S. Genetic diversity of Gorontalo local cattle based on microsatellite DNA. *Advances in Animal and Veterinary Sciences*, **11** (4), 544-551 (2023). DOI: https://dx.doi.org/10.17582/journal.aavs/2023/11.4.544.551

8. Whannou, H. R. V., Spanoghe, M., Dayo, G.-K., Demblon, D., Lanterbecq, D. and Dossa, L. H. Genetic diversity assessment of the indigenous goat population of Benin using microsatellite markers. *Frontiers in Genetics*, **14**,1079048 (2023). DOI: 10.3389/fgene.2023.1079048

9. Odjakova, T., Todorov, P., Radoslavov, G.and Hristov, P. Microsatellite genotyping of two Bulgarian sheep breeds. *Diversity*, **14**, 210 (2022). DOI: https://doi.org/10.3390/d14030210

10. Zhao, P., Zhang, L., Liu, Y., Wang, Z., Guo, H., Li, J., Wu, S., Gao, Z. and Yuan, H. Genetic diversity and phylogenetic relationship estimation of Shanxi indigenous goat breeds using microsatellite markers, *Animal Biotechnology*, **35** (1),1-8 (2023). DOI: 10.1080/10495398.2023.2276717

11. Park, C. S, Lee, S. Y. and Cho, G. J. Evaluation of recent changes in genetic variability in Thoroughbred horses based on microsatellite markers parentage panel in Korea. *Animal Bioscience*, **35** (4), 527-532 (2022). DOI: 10.5713/ab.21.0272

12. Li, Y., Liu, L., Zunongjiang, A., Cao, L., Fan, Y., Hu, B. and Zhang, S. Analysis of the relationship between short tandem repeats and lactation performance of Xinjiang Holstein cows. *Tropical Animal Health and Production*, **55**, 238 (2023). DOI: 10.1007/s11250-023-03651-y

13. Rushdi H. E., Moghaieb R. E. A., Abdel-Shafy, H. and Ibrahim, M. A. M. Association between microsatellite markers and milk production traits in Egyptian buffaloes. *Czech Journal of Animal Science,* **62** (9), 384-391(2017). DOI: 10.17221/80/2016-CJAS

14. Hosseinzadeh, S., Azartash, A., Nikbin, S., Javanmard, A., Ali Abbasi, M., Rafat, S. A., Ghafari, M., Hedayat-Evrigh, N. and Alijani, S. Quantitative trait loci mapping for growth curve variables in Ghezel sheep. *Iranian Journal of Applied Animal Science*, **12** (4), 723-732 (2022).

15. Badbarin, S., Mirhoseini, S. Z., Rabiei, B., Hossein-Zadeh, N. G., Khamisabadi, H., Asroosh, F., Perini, F., Rovelli, G., Seidavi, A. and Lasagna, E. QTLs detection for mohair traits in Iranian Angora goats (Markhoz goats). *Small Ruminant Research*, **202**, 106460 (2021). DOI:10.1016/j.smallrumres.2021.106460

16. Rabie, T. and Soliman, A. Integration of QTL detection of growth traits and association study for chicken chromosome 4. *Egyptian Poultry Science Journal*, **40** (I), 191-207 (2020).

17. Mei, G., Yin, C., Ding, X. and Zhang, Q. Fine mapping quantitative trait loci affecting milk production traits on bovine chromosome 6 in a Chinese Holstein population. *Journal of Genetics and Genomics*, **36**, 653-660 (2009). DOI: 10.1016/S1673-8527(08)60157-5

18. Olsen, H.G., Meuwissen, T. H. E., Nilsen, H., Svendsen, M. and Lien, S. Fine mapping of quantitative trait loci on bovine chromosome 6 affecting calving difficulty. *Journal of Dairy Science*, **91**, 4312-4322 (2008). DOI:10.3168/jds.2008-1000

19. Tsairidou, S., Allen, A. R., Pong-Wong, R., McBride, S. H., Wright, D. M., Matika, O., Pooley, C. M., McDowell, S. W. J., Glass, E. J., Skuce, R. A., Bishop, S. C. and Woolliams, J. A. An analysis of effects of heterozygosity in dairy cattle for bovine tuberculosis resistance. *Animal Genetics*, **49**, 103-109 (2018), DOI:10.1111/age.12637

20. Cai, Z., Wu, X., Thomsen, B., Lund, M. S. and Sahana, G. Genome-wide association study identifies functional genomic variants associated with young stock survival in Nordic Red Dairy Cattle. *Journal of Dairy Science*, **106**, 7832-7845 (2023). DOI: 10.3168/jds.2023-23252

21. Lindholm-Perry, A. K., Sexten, A. K.; Kuehn, L. A., Smith, T. P. L., King, D. A., Shackelford, S. D., Wheeler, T. L., Ferrell, C. L., Jenkins, T. G., Snelling, W. M. and Freetly, H. C. Association, effects and validation of polymorphisms within the NCAPG–LCORL locus located on BTA6 with feed intake, gain, meat and carcass traits in beef cattle. *BMC Genetics*, **12**, 103 (2011). DOI: 10.1186/1471-2156-12-103

22. Iannuzzi, A., Parma, P. and Iannuzzi, L. The Cytogenetics of the water buffalo: A Review. *Animals*, **11** (11), 3109 (2021). DOI: 10.3390/ani11113109

23. Si, J. Dai, D. Li, K. Fang, L. and Zhang, Y. A multi-tissue gene wxpression atlas of water buffalo (*Bubalus bubalis*) reveals transcriptome conservation between buffalo and cattle. *Genes*, **14**, 890 (2023). https://doi.org/10.3390/ genes14040890

24. Khan, M. S. and Chaudhry, H. Z. Prediction of lactation yield from last record day and average daily yield in Nili-Ravi buffaloes. *Buffalo Newsletter*, **15**, 7-10 (2001).

25. Chen, H. Y., Zhang, Q., Yin, C. C., Wang, C. K., Gong, W. J. and Mei, G. Detection of quantitative trait loci affecting milk production traits on bovine chromosome 6 in a Chinese Holstein population by the daughter design. *Journal of Dairy Science*, **89** (2), 782-790 (2006). DOI: 10.3168/jds.S0022-0302 (06)72140-3

26. Ashwell, M. S., Heyen, D. W., Sonstegard, T. S., Van Tassell, C. P., Da, Y., VanRaden, P. M., Ron, M., Weller, J. I. and Lewin, H. A. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. *Journal of Dairy Science*, **87** (2), 468-475 (2004). DOI: 10.3168/jds.S0022-0302(04)73186-0

27. Glaubitz, J. C. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes*, **4**, 309-310 (2004). DOI: https://doi.org/10.1111/j.1471-8286.2004.00597.x

28 Yeh, F. C., Yang, R. C. and Boyle, T. Popgene version 1.31. Microsoft window-based freeware for population genetic analysis. Department of Renewable Resources, University of Alberta Ed., Edmonton, Canada. (1999).

29. Kalinowski, S. T., Taper, M. L. and Marshall, T. C. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16** (5), 1099-1106 (2007). DOI: 10.1111/j.1365-294X.2007.03089.x

30. Sharma, R., Ahlawat, S., Sharma, H., Sharma, P., Panchal, P., Arora, R. and Tantia, M.S. Microsatellite and mitochondrial DNA analyses unveil the genetic structure of native sheep breeds from three major agro-ecological regions of India. *Scientific Reports*, **10** (1), 20422 (2020). DOI: 10.1038/s41598-020-77480-6

31. Abou-Bakr, S., Ibrahim, M. A. M., Hafez, Y. M., Attia, M., Abdel-Salam, S. M. and Mekkawy, W. Genetic characteristics of Egyptian buffalo using DNA microsatellite markers. *Egyptian Journal of Animal Production*, **49** (2), 121-126 (2012).

32. Ünal, E. Ö., Işık, R., Şen, A., Geyik Kuş, E. and Soysal, M. İ. Evaluation of Genetic Diversity and Structure of Turkish Water Buffalo Population by Using 20 Microsatellite Markers. *Animals*, **11** (4), 1067 (2021). DOI:10.3390/ani11041067

33. Khade, K. A., Panigrahi, M., Ahmad, S. F., Kumar, P. and Bhushan, B. Genetic characterization and assessment of diversity in Pandharpuri buffalo breed of India using heterologous microsatellite markers. *Animal Biotechnolog*, **31**, 426-431 (2019). DOI: 10.1080/10495398.2019.1612757

34. Singh, N. P., Vohra, V., Das, R., Verma, U., Tantia, M. S. and Kataria, R. S. Elucidating the genetic diversity using SSR based markers in Gojri buffalo. *Indian Journal of Animal Sciences*, **89** (5), 522-527 (2019).DOI:https://doi.org/10.56093/ijans.v89i5.90019

35. Miluchová, M., Gábor, M. and Gašper, J. Analysis of the Genetic Structure of Slovak Holstein Cattle Using Seven Candidate Genes Related to Milk Quality. *Diversity*, **14**, 989 (2022). DOI: https://doi.org/10.3390/d14110989

36. Jaayid, T.A. and Dragh, M.A. Genetic Biodiversity in Buffalo Population of Iraq Using Microsatellites Markers. *Journal of Agricultural Science and Technology A*, **3**, 297-301 (2013).

37. Darestani, Y. M., Ashtiani, S. R. M. and Sadeghi, M. Study of genetic diversity of Iranian indigenous buffalo populations using microsatellite markers. *Genetika*, **51**, 147-1552019 (2019). DOI: https://doi.org/10.2298/GENSR1901147D

38. Acosta, A., Uffo, O., Sanz, A., Obregón, D., Ronda, R., Osta, R., Martin-Burriel, I., Rodellar, C. and Zaragoza, P. Genetic characterization of the Cuban water buffalo population using microsatellite DNA markers. *Buffalo Bulletin*, **33** (1), 101-106 (2014).

39. Marrero, N. M., Christina da Silva, E., Valdés, A. M., Ponce, G., Benício da Silva, R. C., Barbosa, S. B. P., Reinosa, O. U. and Filho, M. A. G. Genetic relationships between Cuban and Brazilian buffaloes (*Bubalus bubalis*) by microsatellite markers. *Revista de Salud Animal*, **37** (3), 152-163 (2015).

40. Hussain, T., Babar, M. E., Ali, A., Nadeem, A., Rehman, Z. U., Musthafa, M. M. and Marikar, F. M. Microsatellite based genetic variation among the buffalo breed populations in Pakistan. *Journal of Veterinary Research*, **61**, 535-542 (2017). DOI: 10.1515/jvetres-2017-0057

41. Gahlyan, R. K., Vohra, V., Chhotaray, S. and Kataria, R. S. Diversity assessment of a lesser known buffalo population from Central India and its comparative evaluation reveals presence of sufficient genetic variation and absence of selection, *Animal Biotechnology*, **35** (1), 2305550 (2024). DOI: 10.1080/10495398.2024.2305550

42. Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, **32**, 314-331 (1980).

43. El-Kholy A. F., Hoda, Z. H., Amin, A. M. S. and Hassanane, M. S. Genetic diversity in Egyptian buffalo using microsatellite markers. *Arab Journal of Biotechnology*, **10** (2), 219-232 (2007).

44. Uffo, O., Martínez, N., Acosta, A., Sanz, A., Martín-Burriel, I., Osta, R., Rodellar, C. and Zaragoza, P. Analysis of microsatellite markers in a Cuban water buffalo breed. *Journal of Dairy Research*, **84** (3), 289-292 (2017). DOI: 10.1017/S0022029917000425

45. Popa, D. C., Dudu, A., Georgescu, S. E., Burcea, A., Popa, R. A. and Vidu, L. Analysis of genetic diversity of Romanian buffalo-A preliminary study. *Romanian Biotechnological Letters*, **25**, 1418-1423 (2020). DOI: 10.25083/rbl/25.2/1418.1423.

46. Merdan, S. M., El-Zarei, M. F., Ghazy, A. A., Ayoub, M. A., Al-Shawa, Z. M. and Mokhtar, S. A. Genetic diversity analysis of five Egyptian buffalo populations using microsatellite markers. *Journal of Agricultural Science*, **12** (4), 271-282 (2020). DOI:10.5539/jas.v12n4p271

47 Aminafshar, M., Amirinia, C. and Torshizi, R.V. Genetic diversity in buffalo population of Guilan using microsatellite markers. *Journal of Animal and Veterinary Advances*, **7** (11), 1499-1502 (2008).

48. Attia, M., Abou-Bakr, S. and Nigm, A. Genetic differentiation and relationship among Egyptian Nile Delta located buffalo using microsatellite markers. *Egyptian Journal of Animal Production*, **51** (2), 71-77 (2014).

49. Ron, M., Kliger, D., Feldmesserm, E., Seroussi, E., Ezra, E. and Weller, J. I. Multiple quantitative trait locus analysis of bovine chromosome 6 in the Israeli Holstein population by a daughter design. *Genetics*, **159** (2), 727-735 (2001). doi: 10.1093/genetics/159.2.727

50. Olsen, H. G., Lien, S., Gautier, M., Nilsen, H., Roseth, A., Berg, P. R., Sundsaasen, K. K., Svendsen, M. and Meuwissen, T. H. Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. *Genetics*, **169** (1) 275-283 (2005). DOI: 10.1534/genetics.104.031559

51. Mekkawy, W., Hafez, Y.M., Attia, M., Abdel-Salam, S. A. M. and Abou-Bakr, S. Association analysis between microsatellite DNA markers and milk yield and its components in Egyptian buffaloes using random regression model. *Egyptian Journal of Animal Production*, **49** (1), 9-18 (2012).

52. Rushdi, H. E. Molecular analysis of microsatellites associated with milk yield and composition in Egyptian buffalo. *Journal of Animal and Poultry Production*, **9** (4), 227-233 (2018).

53. Vani, A., Kumar, S., Kumar, S., Chauhan, A., Sahoo, N. R., Verma, M. R., Tomar, A. K. and Kumar, P. Exploration of allelic variants in short tandem repeats (STRs) flanking milk production QTLs and their association with milk production traits in Indian water buffaloes. *Tropical Animal Health and Production*, **54** (4), 222 (2022). DOI: 10.1007/s11250-022-03215-6

54. Ashwell, M. S., Heyen, D. W., Weller, J. I., Ron, M., Sonstegard, T. S., Van Tassell, C. P., and Lewin, H. A. Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein-Friesian cattle. *Journal of Dairy Science*, **88** (11), 4111-4119 (2005). DOI: 10.3168/jds.S0022-0302 (05)73095-2

55. Silva, A. A., Azevedo, A. L. S., Verneque, R. S., Gasparini, K., Peixoto, M. G. C. D., da Silva, M. V. G. B., Lopes P. S., Guimarães, S. E. F. and Machado, M. A. Quantitative trait loci affecting milk production traits on bovine chromosome 6 in zebuine Gyr breed. *Journal of Dairy Science*, **94** (2), 971-980 (2011). DOI: 10.3168/jds.2009-2970

56. Chen, H., Zhang, Q., Wang, C., Shu, J., Mei, G., Yin, C., Hu, F., Xu, J., Gong, W., Li, H. and Qiu, X. Mapping QTLs on BTA6 affecting milk production traits in a Chinese Holstein population. *Chinese Science Bulletin*, **50** (16), 1737-1742 (2005). DOI: 10.1360/982005-725

57. Attar, A., Rahimi, G., Nassiri, M. R. and Sadeghi, B. Analysis association of milk fat and protein percent in quantitative trait locus (QTLs) on chromosomes 1, 6, 7 and 20 in Iranian Holstein cattle using ten micro satellite markers. *African Journal of Biotechnology*, **13** (20), 2010-2014 (2014). DOI: 10.5897/AJB10-928

58. Ashwell, M. S., Van Tassell, C. P., and Sonstegard, T. S. A genome scan to identify quantitative trait loci affecting economically important traits in a U.S. Holstein population. *Journal of Dairy Science*, **84** (11), 2535-2542 (2001). DOI: 10.3168/jds.S0022-0302(01)74705-4

59. Olsen, H. G., Lien, S., Svendsen, M., Nilsen, H., Roseth, A., Aasland, O. M. and Meuwissen, T. H. E. Fine mapping of milk production QTL on BTA6 by combined linkage and linkage disequilibrium analysis. *Journal of Dairy Science*, **87** (3), 690-698 (2004). DOI: 10.3168/jds.S0022-0302(04)73212-9.

60. Thomasen, J. R., Guldbrandtsen, B., Sørensen, P., Thomsen, B. and Lund, M. S. Quantitative trait loci affecting calving traits in Danish Holstein cattle. *Journal of Dairy Science*, **91** (5), 2098-2105 (2008). DOI: 10.3168/jds.2007-0602

61. Wang, Y., Nana, Z., Zhanbin, W., Qingyi, W., Xiaohui, Z., Junyan, B. and Youzhi, P. Association of polymorphism of microsatellite markers with litter size in Chinese Funiu white goat. Research *Journal of Animal Sciences*, **4** (3), 92-98 (2010). DOI:10.3923/rjnasci.2010.92.98

62. El-Halawany, N., Abdel-Shafy, H., Abd-El-Monsif, A. S., Abdel-Latif, M. A., Al-Tohamy, A. F. and El-Moneim, O. M. A. Genome-wide association study for milk production in Egyptian buffalo. *Livestock Science*, **198**, 10-16 (2017). DOI: https://doi.org/10.1016/j.livsci.2017.01.019

63. Abdel-Shafy, H., Awad, M. A. A., El-Regalaty, H., El-Assal, S. E-D. and Abou-Bakr, S. Prospecting genomic regions associated with milk production traits in Egyptian buffalo. *Journal of Dairy Research*, **87** (4), 389-396 (2020). DOI: 10.1017/S0022029920000953

64. Awad, M. A. A., Abou-Bakr, S., El-Regalaty, H., El-Assal, S. E-D. and Abdel-Shafy, H. Determination of potential candidate genes associated with milk lactose in Egyptian buffalo. *World's Veterinary Journal*, **10** (1), 35-42 (2020). DOI:10.36380/scil.2020.wvj5

65. EL Nagar, A. G., Salem, M. M. I., Amin, A. M. S., Khalil, M. H., Ashour, A. F., Hegazy, M. M. and Abdel-Shafy, H. A Single-step genome-wide association study for semen traits of Egyptian buffalo bulls. *Animals*, **13** (24), 3758 (2023). DOI: 10.3390/ani13243758

66. de Camargo, G. M., Aspilcueta-Borquis, R. R., Fortes, M. R., Porto-Neto, R., Cardoso, D. F., Santos, D. J., Lehnert, S. A., Reverter, A., Moore, S. S. and Tonhati, H. Prospecting major genes in dairy buffaloes. *BMC Genomics*, **16**, 872 (2015). DOI: 10.1186/s12864-015-1986-2

67. Hosseini, S.M., Tingzhu, Y., Pasandideh, M., Liang, A., Hua, G., Farmanullah, Schreurs, N. M., Raza, S. H. A., Salzano, A., Campanile, G., Gasparrini, B. and Yang, L. Genetic association of PPARGC1A gene single nucleotide polymorphism with milk production traits in Italian Mediterranean buffalo. *BioMed Research International*, **2021**,3653157 (2021). DOI: 10.1155/2021/3653157

68. Vohra, V., Chhotaray, S., Gowane, G., Alex, R., Mukherjee, A., Verma, A. and Deb, S. M. Genome-wide association studies in Indian Buffalo revealed genomic regions for lactation and fertility. Frontiers in *Genetics*, **12**, 696109 (2021). DOI: 10.3389/fgene.2021.696109

69. Kumar, D. R., Nandhini, P. B. , Devadasan, M. J., Sivalingam, J., Mengistu, D. W., Verma, A., Gupta, I. D., Niranjan, S. K., Kataria, R. S. and Tantia, M. S. Genome‐wide association study revealed suggestive QTLs for production and reproduction traits in Indian Murrah buffalo. *Biotech*, **13** (3), 100 (2023). DOI: 10.1007/s13205-023-03505-2

إرتباط واسمات التوابع الدقيقة علي الكروموسوم السابع مع صفات إنتاج اللبن والخصوبة في الجاموس المصري

محمد عطية أحمد عوض و سامي أبوبكر

قسم الإنتاج الحيواني - كلية الزراعة - جامعة القاهرة - الجيزة 12613- مصر

الملخص

تهدف هذه الدراسة إلي دراسة الإرتباط بين 16 من واسمات التوابع الدقيقة الموجودة علي الكروموسوم السابع وصفات إنتاج اللبن والخصوبة في الجاموس المصري. تم تجميع البيانات من 995 سجل حليب لـ 296 من الجاموس المصري خلال الفترة ما بين 2000 إلي 2022. كانت صفات إنتاج اللبن تحت الدراسة هي: إنتاج اللبن الكلي ، إنتاج اللبن في 305 يوم ، طول فترة الحليب ، إنتاج اللبن اليومي ، و طول فترة الجفاف ، بينما كانت صفات الخصوبة هي : عدد التلقيحات اللازمة للحمل ، أول تلقيحة بعد الولادة ، الأيام المفتوحة ، و الفترة ما بين ولادتين. أظهرت جميع الواسمات درجة عالية من تعدد الصور الأليلية ، كان متوسط تقديرات المقاييس الجزيئية الوراثية لواسمات التوابع الدقيقة تحت الدراسة هي: 8.750 ، 7.306 ، 0.857 ، 0.855 ، 0.741 ، 2.033 ، -0.071 للعدد الملاحظ من الأليلات ، العدد الفعال من الأليلات، قيمة الخلط الوراثي الملاحظ ، قيمة الخلط الوراثي المتوقع ، محتوي التنوع الوراثي ، دليل شانون للتنوع ، و معامل التربية الداخلية علي الترتيب. أظهر تحليل الإرتباط أن 14 من الواسمات من أصل 16 قد أظهروا إرتباطات معنوية مع صفات إنتاج اللبن والخصوبة تحت الدراسة ($P < 0.05$, $P < 0.01$, and $P < 0.001$) ، تحديداً سبعة من الواسمات (BM415, BM143, BMC4203, BMS483, RM28, ILSTS93, and ILSTS97)كانت مرتبطة معنوياً مع صفات إنتاج اللبن ، ثلاثة واسمات (BMS2508, BM4311, and BP7) كانت مرتبطة معنوياً مع صفات الخصوبة بينما أظهرت أربعة واسمات (BMS2460, BM1329, BM1236, and AFR227) إرتباطات معنوية مع صفات إنتاج اللبن والخصوبة معاً. تسلط نتائج هذه الدراسة مزيداً من الضوء للكشف عن الخلفية الوراثية المتحكمة في صفات إنتاج اللبن والخصوبة في الجاموس المصري. كما أن الإرتباطات المعنوية بين واسمات التوابع الدقيقة مع الصفات المظهرية تحت الدراسة توفر الدليل علي أن الكروموسوم السابع للجاموس المصري يحمل العديد من المواقع الوراثية التي يحتمل تأثيرها علي التبان الوراثي ، بالإضافة إلي العديد من الجينات المرشحة للتأثير علي تلك الصفات الإقتصادية في الجاموس المصري.

الكلمات الدالة: الإرتباط ، الجاموس الصري ، الخصوبة ، التوابع الدقيقة ، صفات اللبن.