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Improving milk yield and fertility traits in Egyptian buffalo by identifying the functional genes and using genomic approach

By

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LIST OF ABBREVATIONS

Symbol	Abbrevations
AFC	Age at first calving
AM	Animal model
APRI	Animal Production Research Institute
AS	Abnormal sperms
BW	Birth weight
c^2	Proportions of maternal common environmental effects
CI	Calving interval
CN	Chromosome number
CV %	Coefficients of variation
DG	Daliy gain weight
DGAT1	Diacylglycerol O-acyltransferase 1
DIM	Days in milk
DO	Days open
EG	El-Gimmeza
EN	El-Nubariya
ES	El-Serw
EV	Ejaculate volume
FSHR	Follicle-stimulating hormone receptor
GH	Growth hormone gene
GLSM	Generalized least square means
GWAS	Genome-wide association studies
He	Expected heterozygosities
Ho	Observed heterozygosities
HWE	Hardy-Weinberg equilibrium
IMTC	International Livestock Management Training Center at Sakha
LS	Live sperms
MAS	Marker assisted selection
MM	Mahalet Mousa
MS	Motility of sperms
NA	Not available
Ne	The effective number of alleles
NG	El-Nattafe El-Gadid
NK	El-Nattafe El-Kadim

p^2	Proportions of permanent environmental effects
PBVs	Predicted breeding values
PCR	Amplification by polymerase chain reaction
PEV	Prediction error variance
PIC	polymorphic information content
PRL	prolactin gene
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphisms
RRM	Random regression models
S	Sids
SC	Sperms concentration
SD	Standard deviations
SE	Standard error
*SNP*s	Single Nucleated Polymorphism
STR	Short Tandem Repeats
TD	Test-day
TDFY	Test-day fat yield
TDMY	Test-day milk yield
TDPY	Test-day protein yield
TDSCS	Test-day somatic cell score
WW	Weaningweight

1. INTRODUCTION

The domestic water buffalo is the second dairy species of the world after dairy cattle, accounting for about 15% of the total milk production (**FAOSTAT, 2023**). In the 7th century, the Arabs tamed and brought the water buffalo, more especially the river buffalo subspecies, to Egypt and Italy **(Minervino *et al*., 2020)**. The Egyptian buffalo contributes significantly to milk and meat production efficiency, ranking fourth in the world for milk output and sixth for meat production (**FAOSTAT, 2023**; "https://www.fao.org/statistics/en"). In the perspective of global agriculture, the rankings emphasise the Egyptian buffalo's perceived production and efficiency.

In lactation traits of buffalo, test-day milk yield (TD) has been used in the genetic evaluation of breeding animals for milk, fat and protein yields in several countries particularly in Egypt (**El-Bramony *et al*., 2004 and 2017; Amin *et al*., 2015**), in Italy (**Costa *et al*., 2020**), in India (**Sahoo *et al*., 2014; Singh *et al*., 2015 and 2016),** in Brazil **(Tonhati *et al*., 2008; Aspilcueta-Borquis *et al*., 2012**), in Colombia (**Hurtado-Lugo *et al*., 2009**) and in Iran (**Madad *et al*., 2013**). Using TD milk yield parameters, ruled out the need to extend the lactation period to the standard 305 days length. The TD model allows better modeling for genetic and phenotypic trends because it considers the specific effects of TD, *i.e.* the environmental effects are accurately modeled (**Ptak and Schaeffer, 1993; Schaeffer et al., 2000; Nigm et al., 2003**) and the genetic parameter estimates are expected to be more accurate (**Swalve, 1995**). Precise methodology has been proposed to estimate the (co) variance structure among TD records using the Random Regression Model (RRM; **Meyer, 1998**), *i.e.* RRM can be used for TD milk traits which are expressed repeatedly. In addition to the significance of TD recording, the substantial assessment of the estimated breeding values is an essential step in genetic improvement programs (**Meyer, 2004**). Accordingly, the package of BLUPF90 software (**Misztal *et al*., 2018**; http://nee.ads.uga.edu//wiku///doku.php) has become the worldwide

remarkable standard methodology for predicting the breeding values (PBVs) for TD lactation traits and reproduction performance using the repeatability animal model.

For growth traits in buffalo, an ultimate goal in buffalo breeding is to rank the breeding animals according to their genetic merit for the relevant growth traits and use them efficiently in breeding programs. The genetic evaluation of buffalo calves is, therefore, a key issue to identify the superior genetic calves in a herd. Assessment of the predicted breeding values (PBVs) is an essential step for genetic improvement programs in buffalo (**Meyer, 2004**). In evaluating the breeding programs in Egyptian buffalo, the genetic parameters for growth traits (*i.e.* heritability and PBV) are needed to be evaluated accurately to predict the genetic and phenotypic trends for the traits of concern and consequently to evaluate accurately the breeding programs of Egyptian buffalo using the package of BLUPF90 software (**Misztal *et al.*, 2018**; http://nee.ads.uga.edu//wiku///doku.php).

Studies of genetic and phenotypic trends for milk, fat, and protein yields in Egyptian and non-Egyptian buffalo have shown irregular routes. In Egyptian buffalo studies, favorable increases in both genetic and phenotypic trends for milk, fat and protein yields were reported (**El-Arian *et al.*, 2012; Ahmad *et al.*, 2017; Abo-Gamil *et al.*, 2017; EL-Hedainy *et al.*, 2020**), while **Amin *et al.* (2015)** has shown an increase in the genetic trend accompanied by a decrease in the phenotypic trend. Also, most of the non-Egyptian buffalo studies have shown that the genetic and phenotypic trends for milk, fat and protein yields were increasing together (**Pawar *et al.*, 2018; Kour and Narang, 2021**), while some other studies revealed increases or decreases in the genetic trend (**Seno *et al.*, 2010; Aspilcueta-Borquis *et al.*, 2015 ; Nazari *et al.*, 2021**). Regarding the reproduction traits in buffalo, the genetic and phenotypic trends exhibited favorable decreasing trends in age at first calving (AFC), days open (DO) and calving interval (CI) of Egyptian buffalo (**Shalaby *et al.*, 2016** and **Amin *et al.*, 2021**) or non-favorable increasing trends in AFC and CI as reported by **Gupta *et al.* (2015)** and **Kour and Narang (2021)** for Murrah buffalo. Most of the Egyptian research

articles showed that the genetic and phenotypic trends of body weights were favorable positive and showing an increase in both trends for Egyptian buffalo (**El-Bramony, 2014** and **Salem *et al*., 2020**) and were like those of the Indian buffalo (**Malhado *et al*., 2007** and **Gupta *et al*., 2015**). As stated by some Egyptian buffalo studies **(El-Basuini, 2010; Khattab *et al*., 2015; Salem *et al*., 2023)**, heritability estimates for semen traits were mostly low or somewhat moderate and ranged from 0.08 to 0.40 for ejaculate volume, 0.06 to 0.42 for sperms motility, 0.09 to 0.41 for live sperms percentage and 0.46 to 0.49 for sperms concentration. The ranges in breeding values for semen traits in buffalo are high, being -0.45 to 3.32 *ml* for ejaculate volume, -4 % to 52 % for sperms motility, -5.8 to 8.1 % for live sperms and 799 to 1959×10^9 for sperms concentration (**El-Basuini, 2010; Kumar *et al*., 2023)**. Additionally, the studies concerning the genetic and phenotypic trends for semen traits in buffalo are scarce. However, **Kumar *et al*. (2023)** reported that the genetic and phenotypic trends were positive and showing favorable increase in ejaculate volume and sperms motility in Indian buffalo bulls**.**

Domestic water buffalo (*Bubalus bubalis*) are classified into two classes as river buffalo (*Bubalus bubalis bubalis*) which has 50 chromosome and swamp buffalo (*Bubalus bubalis carabanesis*) which has 48 chromosome (**Mishra *et al*., 2015**). The molecular studies for populations of buffalo in terms of DNA sequencing, *SNP*s and PCR-RFLP techniques, computer software and bioinformatics methodology have been facilitated to identify the molecular markers and candidate genes controlling lactation and reproduction traits, growth and semen traits in buffalo **(Gil *et al*., 2013; Abo Al-Ela *et al*., 2014; Gafer *et al*., 2015; Hasanain *et al*., 2016; Freitas *et al*., 2016; Nadeem and Maryam, 2016; Darwish *et al*., 2016; Mavi *et al*., 2017; Kumari *et al*., 2018; Kathiravan *et al*., 2019; Al-Shawa *et al*., 2019; Wang *et al*., 2020; Deshmukh *et al*., 2021; Erdoğan *et al*., 2021; El-Magd *et al*., 2015, 2021; Erdoğan *et al*., 2021; EL Nagar *et al*., 2023)**. Accordingly, these molecular markers could be used in marker assisted selection programs to improve the selection response of lactation and reproduction traits in buffalo. The improvement of reproduction performance in buffaloes by

traditional selection programs is difficult task, due to long generation interval and low heritability estimates for reproduction traits (**Freitas *et al.*, 2016**). In Egyptian buffalo, the reproduction efficiency is greatly influenced by infertility disorders such as anestrus, inactive ovaries and repeat service **(Sosa *et al.*, 2015)**.

In the last decade, the molecular characterizations for some functional candidate genes were identified in different buffalo studies. Among the various genes, prolactin gene (*PRL*) was mapped on chromosome number 2 **(Hu *et al.*, 2009; Lü *et al.*, 2011)** and this gene was molecularly characterized by **Ishaq *et al.* (2013)** and **Nadeem and Maryam (2016)** in Nili-Ravi buffalo, by **Mavi *et al.* (2017)** in Murrah buffalo**,** by **Konca and Akyüz (2017)** and **Özşensoy (2018)** in Anatolian water buffalo, and by **Hasanain *et al.* (2016, 2017)** in Egyptian buffalo. On the other hand, Diacylglycerol O-Acyltransferase 1 gene (*DGAT1)* was mapped on chromosome number 15 and this gene was molecularly characterized in Anatolian buffalo **(Özdil and Ilhan, 2012)**, in Murrah buffalo **(Gil *et al.*, 2013; Freitas *et al.*, 2016** and **Sulabh *et al.*, 2018)**, in Mediterranean buffalo (**Silva *et al.*, 2016)** and Iraqi buffalo **(Kadhim and Ibrahim, 2019)**. Also, Follicle-stimulating hormone receptor gene (*FSHR*) was mapped on chromosome number 12 and it was molecularly investigated in Egyptian buffalo **(Othman and Abdel-samad, 2013; Ramadan *et al.*, 2020; Fouda *et al.*, 2021).** Moreover, growth hormone gene (*GH*) is located on chromosome number 3 and the structure of this gene in buffalo species was unknown (**Andreas *et al.*, 2010; Konca and Akyüz, 2017; Ahmadzadeh *et al.*, 2019; Özkan Ünal *et al.*, 2020** and **Nafiu *et al.*, 2020)**. Some studies were performed to characterize molecularly this gene in Egyptian buffalo by **Othman *et al.* (2012)**, Anatolian water buffalo by **Konca and Akyüz (2017)**, and in Simeulue buffalo by **Eriani *et al.* (2019)**.

On worldwide, the molecular buffalo studies have shown that *PRL, DGAT1, FSHR* and *GH* genes could be used as candidate genes in the genetic improvement programs for lactation and reproduction traits of buffalo in Pakistan **(Nadeem and Maryam, 2016)**, in China **(Li *et al.* 2017)**, in Turkey

(Konca and Akyüz, 2017; Özşensoy, 2018) , and in India **(Mavi *et al.* 2017)**. Also, the Egyptian buffalo molecular studies verified that *PRL*, *DGAT1*, *FSHR* and *GH* genes are considered as important candidate genes that are molecularly associated with milk yields and compositions, reproduction and fertility, semen, body weights and gains in Egyptian buffalo **(Othman and Abdel-samad, 2013; Sosa *et al*, 2015; El-Komy *et al*, 2020; Ramadan *et al*., 2020; Fouda *et al*., 2021; Sallam *et al*., 2022)**. However, *PRL* gene is known to have various biological functions such as water and electrolyte balance, growth, development, immunity and reproduction function (**Gregerson, 2006**). Also, *PRL* gene plays a central role in mammalian reproduction, glandular development, milk secretion, and expression of milk protein. In Murrah buffalo**, Singh *et al*. (2016)** found that *PRL* gene is an important candidate gene known to be associated with milk production traits as well as somatic cell counts (SCC). The Egyptian studies have shown that *FSHR* gene is considered as an important candidate gene for lactation, reproduction, fertility and semen traits in Egyptian buffalo **(Othman and Abdel-samad, 2013; Shafik *et al*., 2017; Ramadan *et al*., 2020; Fouda *et al*., 2021; Sallam *et al*., 2022)**. **Shafik *et al*. (2017)** found significant association between *FSHR* gene and calving interval, days open, days in milk, total milk yield and 305-day milk yield. Regarding *GH* gene, this gene can be used as a candidate gene for the genetic improvement of growth traits in buffalo since it is known to have various biological functions such as water and electrolyte balance, milk production and reproduction functions (**Othman and Abdel-samad, 2013; Darwish *et al.*, 2016)**. Therefore, *GH*, *PRL* and *FSHR* genes proved to be growth encouraging factors and can serve as candidate genes to identify the molecular markers associated with lactation, reproduction, semen and growth traits for selection programs in buffalo. Yet, studies on the variability and association among these candidate genes and lactation, reproduction, semen and growth traits still limited in Egyptian buffalo. So, the main objectives of the present study were: 1) evaluate genetically some lactation traits [Test-day milk yield (TDMY), Test-day fat yield (TDFY), Test-day protein yield (TDPY) and

Test-day somatic cell score (TDSCS)], reproduction traits [age at first calving (AFC), days open (DO) and calving interval (CI)], semen traits [ejaculate volume (EV), motility of sperms (MS), live sperms (LS), abnormal sperms (AS) and sperms concentration (SC)] and growth traits [birth weight (BW), weaning weight (WW) and daily gain weight (DG)] in Egyptian buffalo through estimating the variance components and heritability estimates using Bayesian Gibbs Sampling Algorithm, 2) predict the breeding values and plot the genetic and phenotypic trends for these traits using BLUPF90 software, 3) to characterize on *SNPs* basis the *PRL, DGAT1*, *FSHR* and *GH* genes in Egyptian buffalo populations, 4) to use PCR-RFLP technique in genotyping the *SNP* genotypes located in the promoter regions of these genes, and 5) to detect the molecular associations among *SNP* genotypes of *PRL, FSHR* and *GH* genes and lactation, reproduction, semen and growth traits in Egyptian buffalo using PCR-RFLP technique and generalized least square means proudure (GLSM).

2. REVIEW OF LITERATURE

2.1 Reviewed heritabilities for test-day milk yield and components estimated by random regression model and animal model

The estimates of variance components and heritabilities for test-day milk yields and components obtained by random regression model (RRM) were firstly published by **Jamrozik and Schaeffer (1997)**. **Meyer and Hill (1997)** and **Meyer (1998)** demonstrated that the use of covariance functions is necessary for the model including the additive genetic and permanent environmental effects in the random regression model of test day (TD). The RRM as used for analysing lactation traits like test-day milk yields and components which are expressed repeatedly with the inclusion of fixed regressions on days in milk (DIM) in the model of analysis, *i.e.* different regression coefficients of DIM for each animal could be estimated. According to **Tonhati *et al*. (2008),** the adoption of test-day milk yields (TDMY) as selection criteria in milking buffalo may contribute to greater genetic gain in total milk production. Many studies were published for Brazilian dairy cattle evaluating different RRM for test-day models **(El Faro & Albuquerque 2003; Costa *et al*. 2005; Araújo *et al*. 2006; El Faro *et al*. 2008; Bignardi *et al*. 2009; Cobuci *et al*. 2011; Herrera *et al*. 2013)**. Also, RRM are currently being used for national genetic evaluations of dairy cattle in several countries. In general, genetic analyses for milk yield in buffalo have been carried out using finite dimensional models **(Rosati & Van Vleck 2002)**; however, there are limited studies about applying RRM to estimate genetic parameters for buffaloes TDMY. Thus, it is crucial to develop these models to be implemented in a genetic evaluation programme for milking buffalo in Brazil.

Random regression models (RRM) have been proposed as an alternative methodology for the analysis of longitudinal data or repeated measures records (**Sesana *et al*, 2010**). Therefore, the random regression models were recommended for the analyses of test-day models in dairy cattle due to the following reasons: 1) the RRMs allow to obtain the breeding values for milk yield at any day of lactation in a continuous manner or

functions of the lactation curve, 2) the RRMs allow estimates of covariances between coefficients of random functions or equivalently the estimates of covariance functions, 3) the RRMs provide estimates of breeding values with higher accuracies than the conventional finite dimensional models because all records available from lactation and short length lactation records can be used in the genetic evaluation (**Jamrozik *et al.* 2000; Schaeffer *et al.* 2000**), 4) By applying the RRMs, additive genetic and permanent environmental effects could change the average shape of lactation curve (**Strabel *et al.*, 2005**), 5) With RRM, a structure on the covariance matrices can be imposed and the latter give the covariance between any two records along the lactation curve (**Meyer 1998a**). In dairy cattle, the choice of covariance function order for additive genetic and permanent environmental effects is the focus in finding an optimal RRM (**Liu *et al.* 2006**). Moreover, for the analyses of Test-Day lactation records, RRM is the model of choice due to the following characteristics: 1) Random regressions allow for a different shape of lactation curves. (**Amin, 2006**). 2) The RRM also allows a cow to be evaluated on the basis of any number of TD records during lactation and it can account for different genetic, permanent environmental and residual variances at different stages of lactation, thus resulting in more reliable genetic evaluation. (**Amin, 2006**). 3) RRM are more appropriate for estimating the genetic parameters of test-day milk yield than repeatability models, because random regression models are able to fit genetic and environmental changes in milk yield over the time. 4) RRM provide insights about temporal variation of biological processes and physiological implications underlying the studied traits. 5) RRM can accommodate changing residual variances throughout lactation, such as higher variability in early or late lactation.

The monthly test-day milk yield and components were considered with an interval of 30 days. Many researchers have evaluated the lactation traits of buffalo based on genetic bases using monthly test day milk yield and components (**El-Bramony *et al.*, 2004; Geetha *et al.*, 2006; Hurtado-Lugo *et al.*, 2006&2009; Aspilcueta-Borquis *et al.*, 2007&2010; Madad *et al.*, 2013; Kumar *et al.*, 2014; Amin *et al*, 2015; Singh *et al.*, 2016)**. The

heritability estimates for test-day milk yields and components estimated by animal model and RRM are reviewed and presented in **Table 1&2**. The trend could be notified as most heritability estimates were low at the beginning of lactation, gradually increased reaching the highest value and decreased gradually until reached the lowest value. Most heritability estimates obtained by RRM and animal model in Egyptian buffalo were high at the edges **(Amin *et al.*, 2015)**. As shown in **Table 1**, the ranges in heritability for test-day milk yield in buffalo were 0.04 to 0.39 for TD_1, 0.04 to 0.44 for TD_2, 0.002 to 0.47 for TD_3, 0.03 to 0.4 for TD_4, 0.03 to 0.37 for TD_5, 0.06 to 0.43 for TD_6, 0.09 to 0.39 for TD_7, 0.11 to 0.37 for TD_8 and 0.01 to 0.55 for TD_9. The minimum ranges are reported by **El-Bramony *et al.* (2004)**, while the maximum ranges were reported in the first four TDs by **Hurtado-Lugo *et al.* (2009)**, the subsequent four TD were reported by **Geetha *et al*. (2006)** and the ninth TD by **El-Bramony *et al.* (2004)**. **Aspilcueta-Borquis *et al*. (2012)** in Murrah buffalo found that heritabilities estimated by RRM were 0.16 to 0.29, 0.20 to 0.30, and 0.18 to 0.27 for test-day milk, fat and protein yields, respectively. **Amin *et al.* (2015)** reported definite trend for heritability estimates of milk yield in Egyptian buffalo to be low at the beginning of the test day (0.05 to 0.30) and gradually increased reaching the highest value at the fourth test day reaching 0.28 and 0.31, then the estimates decreased gradually until reaching the lowest value at the tenth test day of lactation (0.06 to 0.10). **El-Bramony *et al.* (2004)** found that heritabilities for test-day somatic cell count in Egyptian buffalo estimated by RRM were 0.04 to 0.11 for TD_1, 0.01 to 0.10 for TD_2, 0.001 to 0.14 for TD_3, 0.01 to 0.15 for TD_4, 0.003 to 0.10 for TD_5, 0.003 to 0.06 for TD_6, 0.003 to 0.15 for TD_7, 0.09 to 0.19 for TD_8 and 0.17 to 0.53 for TD_9.

The variance components and heritabilities for test-day milk yields and components estimated by animal model as cited in buffalo literature are presented in **Table 2. El-Bramony *et al.* (2017)** in Egyptian buffalo found that heritabilities estimated by fitting bivariate repeatability animal model were 0.04 to 0.15, 0.02 to 0.11 and 0.07 to 0.13 for test-day milk, fat and protein yields, respectively. **Aspilcueta-Borquis *et al.* (2010)** in Murrah

buffalo found that heritabilities estimated by animal model for test-day milk yields were 0.13 to 0.23 for single-trait analyses, 0.13 to 0.24 for two-trait analyses, and 0.15 to 0.24 for multiple-trait analyses.

Aspilcueta-Borquis *et al.* (2010) in Murrah buffalo found that heritabilities estimated by animal model were moderate, being 0.33, 0.39, and 0.26 for fat%, protein% and somatic cell score, respectively. In Egyptian buffalo**, Ibrahim *et al.* (2012)** found that heritabilities estimated by multiple-trait animal model were 0.40, 0.19, 0.22 and 0.05 for milk, fat, protein yields and somatic cell score, respectively. In Brazilian buffalo, **De Camargo *et al.* (2015)** reported that heritabilities estimated by animal model were moderate, being 0.25, 0.22, 0.26 and 0.17 for milk, fat, protein yields and somatic cell score, respectively.

Table 1. Heritabilities for test-day milk, fat and protein yields estimated by random regression model as cited in buffalo literature

Reference and country of work	N	Test day of lactation (TD)								
		1	2	3	4	5	6	7	8	9
Test-day milk yield:										
El-Bramony *et al*. (2004), Egyptian buffalo, Egypt	3189	0.05	0.06	0.06	0.04	0.03	0.06	0.23	0.28	0.38
		0.22	0.25	0.24	0.23	0.21	0.12	0.09	0.26	0.34
		0.25	0.04	0.002	0.03	0.08	0.11	0.16	0.33	0.55
Geetha *et al*. (2006), Murrah buffalo, India	791	0.39	0.41	0.42	0.39	0.37	0.43	0.39	0.37	0.33
Hurtado-Lugo *et al*. (2006), Colombian buffalo, Colombia	3154	0.04	0.10	0.04	0.07	0.20	0.16	0.17	0.14	0.01
Aspilcueta-Borquis *et al*. (2007), Murrah buffalo, Brazil	42813	0.19	0.22	0.24	0.19	0.20	0.19	0.11	0.11	0.09
Hurtado-Lugo *et al*. (2009), Colombian buffalo, Colombia	5575	0.39	0.44	0.47	0.40	0.33	0.35	0.38	0.35	0.23
Aspilcueta-Borquis *et al*. (2012) Murrah buffalo, Brazil	1433	0.16	0.22	0.26	0.31	0.29	0.27	0.24	0.20	0.21
Madad *et al*. (2013), Iranian buffalo, Iran	9278	0.33	0.19	0.23	0.27	0.22	0.15	0.09	0.15	0.31
Amin *et al*. (2015) Egyptian buffalo, Egypt	4971	0.05	0.22	0.28	0.30	0.27	0.23	0.18	0.16	0.12
Test-day fat yield:										
Aspilcueta-Borquis *et al*. (2007) Murrah buffalo, Brazil	7008	0.23	0.21	0.17	0.18	0.14	0.08	0.11	0.08	0.09
Aspilcueta-Borquis *et al*. (2012) Murrah buffalo, Brazil	1433	0.22	0.20	0.23	0.24	0.26	0.26	0.23	0.21	0.30
Madad *et al*. (2013) Iranian buffalo, Iran	8050	0.24	0.08	0.11	0.08	0.07	0.16	0.23	0.18	0.03
Amin *et al*. (2015) Egyptian buffalo, Egypt	4971	0.05	0.21	0.26	0.28	0.27	0.19	0.25	0.19	0.15
Test-day protein yield:										
Aspilcueta-Borquis *et al*. (2007) Murrah buffalo, Brazil	7008	0.32	0.32	0.33	0.22	0.11	0.11	0.16	0.04	0.05
Aspilcueta-Borquis *et al*. (2012) Murrah buffalo, Brazil	1433	0.16	0.24	0.25	0.26	0.25	0.24	0.20	0.25	0.27
Madad *et al*. (2013), Iranian buffalo, Iran	1945	0.24	0.26	0.21	0.27	0.25	0.07	0.01	0.04	0.26
Amin *et al*. (2015) Egyptian buffalo, Egypt	4971	0.06	0.20	0.29	0. 31	0.29	0.24	0.15	0.08	0.05

Table 1. Cont.

Reference and country of work	N	Test day of lactation (TD)								
		1	2	3	4	5	6	7	8	9
Test-day somatic cell count:										
El-Bramony *et al*. (2004) Egyptian buffalo, Egypt	3189 (three paritie)	0.05	0.10	0.14	0.15	0.10	0.05	0.07	0.19	0.53
		0.11	0.03	0.01	0.01	0.003	0.003	0.03	0.09	0.17
		0.04	0.01	0.001	0.02	0.04	0.08	0.15	0.18	0.31

Table 2. Heritabilities for test-day milk, fat and protein yields estimated by animal model as cited in buffalo literature

Reference and country of work	N	Test day of lactation (TD)								
		1	2	3	4	5	6	7	8	9
Test-day milk yield:										
Tonhati *et al*. (2008), Murrah buffalo, Brazil	3888	0.23	0.29	0.30	0.26	0.25	0.25	0.19	0.17	0.12
Aspilcueta-Borquis *et al*. (2010), Murrah buffalo, Brazil	4757	0.18	0.20	0.24	0.23	0.22	0.19	0.15	0.13	0.13
Chakraborty *et al*. (2010), Murrah buffalo, India	5216	0.18	0.27	0.20	0.29	0.39	0.34	0.27	0.22	0.16
Sahoo *et al*. (2014), Murrah buffalo, India	3905	0.26	0.28	0.19	0.24	0.19	0.17	0.15	0.16	0.14
Singh *et al*. (2016), Murrah buffalo, India	9071	0.16	0.12	0.18	0.14	0.15	0.09	0.15	0.10	0.12
El-Bramony *et al*. (2017) Egyptian buffalo, Egypt.	7926	0.06	0.05	0.05	0.10	0.11	0.12	0.15	0.04	0.04
Test-day fat yield:										
Aspilcueta-Borquis *et al*. (2010) Murrah buffalo, Brazil	4757	0.18	0.19	0.21	0.21	0.21	0.23	0.22	0.18	0.16
Kumar *et al*. (2016), India	10381	0.28	0.37	0.43	0.30	0.41	0.40	0.29	0.06	0.22
El-Bramony *et al*. (2017) Egyptian buffalo, Egypt.	7926	0.04	0.02	0.02	0.06	0.06	0.08	0.11	0.02	0.02
Test-day protein yield:										
Aspilcueta-Borquis *et al*. (2010) Murrah buffalo, Brazil	4757	0.16	0.18	0.21	0.22	0.21	0.20	0.15	0.15	0.13
El-Bramony *et al*. (2017) Egyptian buffalo, Egypt.	7926	0.07	0.09	0.08	0.09	0.09	0.11	0.13	0.09	0.07

2.2 Reviewed heritabilities for reproduction traits estimated by animal model

The estimates of heritability for reproduction traits as cited in buffalo literature were shown in **Table 3**. These reviewed estimates of heritability were mostly low or rarely moderate, being 0.015 to 0.35 for age at first calving, 0.002 to 0.217 for calving interval, 0.0001 to 0.18 for days open **(Afifi *et al*., 1992; Aziz *et al*., 2001; Catillo *et al*., 2001; Morammazi *et al*., 2007; Suhail *et al*., 2009; Malhado *et al*., 2012; El-Bramony, 2014; Agudelo-Gómez *et al*., 2015; De Camargo *et al*., 2015; Barros *et al*., 2016; Ashmawy and El-Bramony, 2017; Mostafa *et al*., 2017; Shafik *et al*., 2017; Amin *et al*., 2020, 2021; Helmy and Somida, 2021; Kour and Narang, 2021; Easa *et al*., 2022; Kaplan and Tekerli, 2023)**.

Table 3. Reviewed heritabilities (h^2) for reproduction traits estimated by animal model as cited in buffalo literature

Reference and country of research	Breed used	N	h^2±SE
Age at first calving:			
Catillo *et al*. (2001), Italy	Italian buffalo	21098	0.26
Suhail *et al*. (2009), Pakistan	Nili-Ravi buffalo	5037	0.28
Seno *et al*. (2010), India	Murrah buffalo	1578	0.07±0.05
Thiruvenkadan *et al*. (2010), India	Murrah buffalo	698	0.40±0.12
El-Bramony (2011), Egypt	Egyptian buffalo	1911	0.11±0.06
Agudelo-Gómez *et al*. (2015), Colombia	Colombia buffaloes	4138	0.14±0.03
Gupta *et al* (2015), India	Murrah buffalo	1456	0.135±0.035
Kumar *et al* (2015), India	Murrah buffalo	827	0.28±0.03
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	3431	0.17±0.02
Barros *et al*. (2016), Brazil	Murrah buffalo	2389	0.16
Ashmawy and El-Bramony (2017), Egypt	Egyptian buffalo	2085	0.145±0.04
de Araujo Neto *et al* (2020), Brazil	Murrah buffalo	2290	0.16
Amin *et al*. (2021), Egypt	Egyptian buffalo	2426	0.12±0.04
Helmy and Somida (2021), Egypt	Egyptian buffalo	1534	0.12±0.04
Kour and Narang (2021), India	Murrah buffaloes	659	0.015±0.025
Easa *et al*. (2022), Egypt	Egyptian buffalo	907	0.35±0.10

Table 3. Cont.

Reference and country of research	Breed used	N	h^2±SE
Days open:			
Afifi *et al.* (1992), Egypt	Egyptian buffalo	2946	0.03 ± 0.03
Aziz *et al*. (2001), Egypt	Egyptian buffalo	2505	0.08
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	6894	0.14±0.03
Mostafa *et al*. (2017), Egypt	Egyptian buffalo	3499	0.13±0.02
Amin *et al*. (2021), Egypt	Egyptian buffalo	38906	0.0001±0.0.01
Shafik *et al*. (2017), Egypt	Egyptian buffalo	955	0.18 ±0.04
Helmy and Somida (2021), Egypt	Egyptian buffalo	6500	0.0001±0.01
Calving interval:			
Aziz *et al*. (2001), Egypt	Egyptian buffalo	2505	0.07
Catillo *et al*. (2001), Italy	Italian buffalo	94028	0.05
Morammazi *et al*. (2007), Iran	Khuzestan buffalos	146	0.09±0.13
Suhail *et al*. (2009), Pakistan	Nili-Ravi buffalo	5037	0.15 to 0.18
Malhado *et al*. (2012), Brazil	Murrah buffaloes	1721	0.03
El-Bramony, (2014), Egypt	Egyptian buffalo	2066	0.06±0.01
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	4729	0.06±0.01
Barros *et al*. (2016), Brazil	Murrah buffalo	5672	0.05
Ashmawy and El-Bramony (2017), Egypt	Egyptian buffalo	2146	0.05
Shafik *et al*. (2017), Egypt	Egyptian buffalo	955	0.19 ±0.04
Amin *et al*. (2021), Egypt	Egyptian buffalo	38906	0.02±0.02
Helmy and Somida (2021), Egypt	Egyptian buffalo	6500	0.002±0.02
Kour and Narang (2021), India	Murrah buffaloes	659	0.217±0.00
Easa *et al*. (2022), Egypt	Egyptian buffalo	907	0.09±0.06
Kaplan and Tekerli (2023), Turkey	Anatolian buffaloes	493	0.11
Ramadan *et al*. (2023), Egypt	Egyptian buffalo	1563	0.08±0.034

SE= standerd error.

2.3 Reviewed heritabilities for semen traits estimated by animal model

The estimates of heritability for semen traits as cited in buffalo literature were shown in **Table 4**. These reviewed estimates were mostly low and ranged from 0.08 to 0.401 for ejaculate volume, 0.06 to 0.52 for sperms motility, 0.09 to 0.51 for live sperms, 0.04 for abnormal sperms and 0.46 to 0.49 for sperms concentration **(El-Basuini, 2010; Khattab *et al*., 2015; Bhave *et al*., 2020; Salem *et al*., 2023)**. In cattle, the estimates ranged from 0.03 to 0.32 for ejaculate volume, 0.03 to 0.43 for sperms motility, 0.18 to 0.33 for live sperms, 0.25 to 0.39 for abnormal sperms, 0.07 to 0.52 for sperms concentration **(Mathevon *et al*., 1998; Kapš *et al*., 2000; Druet *et al*.,**

2009; El-Komy *et al*., 2016; Carvalho Filho *et al*., 2020; Yin *et al*., 2019; Olsen *et al*., 2020; Khattab *et al*., 2022). However, these estimates varied from one study to another and these differences in semen traits may be due to several factors such as the fixed effects and covariates included in the model, structure of data used, genetic constitution of the buffalo type, and coefficients of inbreeding and the relationship coefficient matrix.

Table 4. Reviewed heritabilities (h^2) for semen traits estimated by animal model as cited in buffalo and cattle literature

Reference and country of work	Breed used	No of records	h^2±SE
In buffalo:			
Ejaculate volume:			
El-Basuini (2010), Egypt	Egyptian buffalo	1149	0.08
Khattab *et al*. (2015), Egypt	Egyptian buffalo	1128	0.30 ±0.08
Bhave *et al*. (2020), India	Indian buffalo (Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri, and Surti)	97023	0.401±0.029
Salem *et al*. (2023), Egypt	Egyptian buffalo	7761	0.08±0.07
Sperms motility:			
El-Basuini (2010), Egypt	Egyptian buffalo	1149	0.06
Khattab *et al*. (2015), Egypt	Egyptian buffalo	1128	0.35±0.08
Bhave *et al*. (2020), India	Indian buffalo (Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri, and Surti)	97023	0.121±0.013
Salem *et al*. (2023), Egypt	Egyptian buffalo	7761	0.52±0.26
Live sperms:			
El-Basuini (2010), Egypt	Egyptian buffalo	1149	0.09
Khattab *et al*. (2015), Egypt	Egyptian buffalo	1128	0.38±0.08
Salem *et al*. (2023), Egypt	Egyptian buffalo	7761	0.51±0.25
Sperms concentration:			
Bhave *et al*. (2020), India	India buffalo	97023	0.463±0.029
Salem *et al*. (2023), Egypt	Egyptian buffalo	7761	0.49±0.245
Abnormal sperms:			
Salem *et al*. (2023), Egypt	Egyptian buffalo	7761	0.04±0.039

Table 4. Cont.

Reference and country of work	Breed used	No of records	h^2±SE
In cattle:			
Ejaculate volume:			
Mathevon *et al*. (1998), Canada	Holstein	5644	0.24
Kapš *et al*. (2000), Bulgaria	Simmental	955	0.04
Druet *et al*. (2009), France	Holstein	515	0.22±0.05
El-Komy *et al*. (2016), Egypt	Friesian	15153	0.32±0.10
Yin *et al* . (2019), China	Chinese Holstein	1450	0.15± 0.03
Olsen *et al*. (2020), Norwegi	Norwegian Red	14972	0.14±0.02
Khattab *et al*. (2022), Egypt	Friesian	14696	0.13± 0.10
Sperms motility:			
Mathevon *et al*. (1998), Canada	Holstein	5644	0.31
Druet *et al*. (2009), France	Holstein	515	0.43±0.08
Carvalho Filho *et al*. (2020), Brazil	Nellore	506	0.07±0.08
Yin *et al* . (2019), China	Chinese Holstein	1450	0.12±0.03
Olsen *et al*. (2020), Norwegian	Norwegian Red	14972	0.03±0.01 to 0.10±0.04
Khattab *et al*. (2022), Egypt	Friesian	14696	0.32±0.06
Live sperms:			
El- Komy *et al*. (2016), Egypt	Friesian	15153	0.18±0.09
Khattab *et al*. (2022), Egypt	Friesian	14696	0.33±0.07
Sperms concentration:			
Mathevon *et al*. (1998), Canada	Holstein	5644	0.52
Kapš *et al*. (2000), Bulgaria	Simmental	955	0.26
Druet *et al*. (2009), France	Holstein	515	0.19±0.05
El-Komey *et al*. (2016), Egypt	Friesian	15153	0.14±0.09
Yin *et al* . (2019), China	Chinese Holstein	1450	0.22±0.04
Olsen *et al*. (2020), Norwegi	Norwegian Red	14972	0.07±0.02 to 0.14±0.06
Khattab *et al*. (2022), Egypt	Friesian	14696	0.29±0.05
Abnormal sperms:			
Druet *et al*. (2009), France	Holstein	515	0.25±0.10
Carvalho Filho *et al*. (2020), Brazil	Nellore	500	0.39±0.15

SE= standerd error.

2.4 Reviewed heritabilities for body weights at birth and weaning

Heritabilities estimated by animal model for body weights at birth (BW) and weaning (WW) as cited in buffalo literature are given in **Table 5**. These reviewed estimates were moderate or high, ranging from 0.19 to 0.62 for birth weight and 0.02 to 0.41 for weaning weight **(Mahdy *et al*., 1999;**

Cassiano *et al*., 2004; EL-Awady *et al*., 2005; Malhado *et al*., 2007; Mourad and Khattab, 2009; Thiruvenkadan *et al*., 2009; Shahin *et al*., 2010; Falleiro *et al*., 2013; Agudelo-Gómez *et al*., 2015; Gupta *et al*., 2015; Ashmawy and El-Bramony, 2017; Elsayed *et al*., 2021; Rezende *et al*., 2020; Salem *et al*., 2020; Easa *et al*., 2022; Gowane *et al*., 2022; Ramadan *et al*., 2023). These estimates indicate that heritability estimates of body weights in Egyptian buffalo were higher than those estimates for exotic buffalo (e.g Italian, Indian, Pakistani and Brazilian) since Egyptian buffalo were not subjected to intensive programmes of selection in Egypt.

Table 5. Reviewed heritabilities for body weights at birth (BW) and weaning (WW) estimated by animal model as cited in buffalo literature

Reference and country of work	Breed used	N	BW	WW
			h^2±SE	h^2±SE
Egyptian studies:				
Mahdy *et al*. (1999)	Egyptian buffalo	2839	0.10	NA
EL-Awady *et al*. (2005)	Egyptian buffalo	5405	0.35±0.03	0.39±0.04
Mourad and Khattab (2009)	Egyptian buffalo	2262	0.046	0.257
Shahin *et al*. (2010)	Egyptian buffalo	244	0.49	0.10
Ashmawy and El-Bramony (2017)	Egyptian buffalo	2146	NA	0.19±0.04
Salem *et al*. (2020)	Egyptian buffalo	8099	0.06±0.03	0.41±0.07
Easa *et al*. (2022)	Egyptian buffalo	907	0.20±0.08	NA
Ramadan *et al*. (2023)	Egyptian buffalo	1563	0.20±0.034	0.10±0.014
Non-Egyptian studies:				
Cassiano *et al*. (2004), Brazil	Murrah buffalo	2884	0.62	NA
Malhado *et al*. (2007), Brazil	Brazilian buffalo	6992	0.09±0.03	NA
Suhail *et al*. (2009), Pakistan	Nili-Ravi buffalo	5037	0.39	NA
Thiruvenkadan *et al*. (2009), India	Murrah buffalo	590	0.12±0.01	0.19±0.02
Falleiro *et al*. (2013), Brazil	Brazilian buffalo	5169	0.30 to 0.31	NA
Agudelo-Gómez *et al*. (2015), Colombia	Colombian buffalo	12479	NA	0.16
Gupta *et al*. (2015), India	Murrah buffalo	725	0.35±0.16	NA
Rezende *et al*. (2020), Italy	Murrah buffalo	4675	0.41	NA
	Mediterranean	405	0.26	
	Jaffarabadi	766	0.17	
Elsayed *et al*. (2021)	Syrian buffalo	501	0.19	0.02
Gowane *et al*. (2022), India	Murrah buffalo	3754	0.19±0.03	0.14±0.05

SE = Standard error; NA = Not available**.**

2.5 Reviewed predicted breeding values (PBVs)

2.5.1 Reviewed predicted breeding values for lactation traits

The estimates of predicted breeding values for milk yield and components traits as cited in buffalo literature were shown in **Table 6**. In Egyptian buffalo, the ranges of the breeding values were -1548 to 1904 kg for milk yield, -85 to 93 kg for fat yield, -47 to 44 kg for protein yield and -1.16 to 8.03 (log^{10}) for somatic cell count **(Khattab and Mourad, 1992; Khattab *et al*., 2003, 2010; Abdel-Salam *et al*., 2009; El-Arian *et al*., 2012; Amin *et al*., 2015; Ahmad *et al*., 2017; Abo-Gamil *et al*., 2017; EL-Hedainy *et al*., 2020)**.

Table 6. Predicted breeding values (PBV) for lactation traits as cited in buffalo literature

Reference and country of research	Breed used	N	Range in PBV
Milk yield (kg):			
Khattab and Mourad (1992), Egypt	Egyptian buffalo	1180	-147 to154
Khattab *et al*. (2003), Egypt	Egyptian buffalo	1226	-263 to 376
Ahmad, (2007), Pakistan	Nili-Ravi buffalo	263	-922 to 2954
Ahmad *et al*. (2008), Pakistan	Nili-Ravi buffalo	1524	-323 to 345
Abdel-Salam *et al*. (2009), Egypt	Egyptian buffalo	3526	-302 to 297
Khattab *et al*. (2010), Egypt	Egyptian buffalo	834	1020
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	-578 to 840
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	11530	169.63
Kumar and Chakravarty, (2016), India	Murrah buffalo	832	1630 to 2022
Ahmad *et al*. (2017), Egypt	Egyptian buffalo	2763	-1548 to 1866
Abo-Gamil *et al*. (2017), Egypt	Egyptian buffalo	1600	-600 to 400
EL-Hedainy *et al*. (2020), Egypt	Egyptian buffalo	1792	-881 to 1904
Fat yield (kg):			
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	-85 to 93
Amin *et al*. (2015), Egypt	Egyptian buffalo	4971	-11.3 to 11.2
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	2890	6.59
Protein yield (kg):			
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	-47 to 44
Amin *et al*. (2015), Egypt	Egyptian buffalo	4971	-6.1 to 7.1
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	2890	5.28
Somatic cell count (log^{10}):			
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	-1.16 to 8.03
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	2890	0.35

2.5.2 Reviewed predicted breeding values for reproduction traits

The estimates of predicted breeding values for reproduction traits as cited in buffalo and cattle literature were shown in **Table 7**. In buffalo, the ranges in the breeding values were -15.8 to 143 day for age at first calving, -43.1 to 97.9 day for days open and -1.6 to 3.7 day for calving interval **(Bashir *et al*., 2009; Agudelo-Gómez *et al*., 2015; De Camargo *et al*., 2015; Shalaby *et al*., 2016; Shafik *et al*., 2017; Abo-Gamil *et al*., 2017)**. In cattle, the ranges in breeding values were -45 to 36 days for AFC, -15 to 12 days for DO and -24 to 25 days for CI **(Amimo *et al*., 2006; Ilatsia *et al*., 2007; Ibrahim *et al*., 2009; Osman *et al*., 2013; Ghiasi and Honarvar, 2016; Rahbar *et al*., 2016; El-Awady *et al*., 2017; Zahed *et al*., 2020; Caivio-Nasner *et al*., 2021; Kgari *et al*., 2023)**.

Table 7. Predicted breeding values (PBV) for reproduction traits as cited in buffalo and cattle literature

Reference and country of research	Breed used	N	Ranges in PBV
In buffalos:			
Age at first calving (day):			
Catillo *et al*. (2001), Italy	Italian buffalo	94028	-2.7
Khattab *et al*. (2003), Egypt	Egyptian buffalo	1226	-1.8 to 6.3
Bashir *et al*. (2009), Pakistan	Nili-Ravi buffalo	2169	-11 to 143
Agudelo-Gómez *et al*. (2015), Colombia	Colombian buffaloes	23947	-5.50 to 1.67
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	3431	3.78
Shafik *et al*. (2017),Egypt	Egyptian buffalo	955	-15.8 to 25.2
Abo-Gamil *et al*. (2017), Egypt	Egyptian buffalo	1600	-10.9 to 25.1
Days open (day):			
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	6894	3.89
Shalaby *et al*. (2016), Egypt	Egyptian buffalo	1779	-5.65 to -3.68
Shafik *et al*. (2017),Egypt	Egyptian buffalo	955	-43.1 to 97.9
Abo-Gamil *et al*. (2017), Egypt	Egyptian buffalo	1600	-42.5 to 97.7
Calving interval (day):			
De Camargo *et al*. (2015), Brazil	Brazilian buffalo	4729	1.28
Shalaby *et al*. (2016), Egypt	Egyptian buffalo	1779	-3.77 to-5.80
Shafik *et al*. (2017), Egypt	Egyptian buffalo	955	-1.6 to 3.7
Abo-Gamil *et al*. (2017), Egypt	Egyptian buffalo	1600	-1.4 to 3.5

Table 7. Cont.

Reference and country of research	Breed used	N	Ranges in PBV
In cattle:			
Age at first calving:			
Amimo *et al*. (2006), Kenya	Ayrshire cattle	2757	-6 to 6
Ibrahim *et al*. (2009), Egypt	Holstein cattle	3656	-7.5 to 6
Osman *et al*. (2013), Egypt	Holstein-Friesian cattle	3460	-9 to 3
Zahed *et al*. (2020), Egypt	Friesian cattle	3625	-7 to 6
Kgari *et al*. (2023), South Africa	Holstein cattle	64464	-45 to 36
Days open:			
Osman *et al*. (2013), Egypt	Holstein-Friesian cattle	3460	-8 to 3
Ghiasi and Honarvar (2016), Iran	Iranian Holstein	72124	-8 to 3
Rahbar *et al* (2016), Iran	Holstein cattle	23402	-1.2 to 3
El-Awady *et al*. (2017), Egypt	Friesian cattle	5728	-8 to 9
Zahed *et al*. (2020), Egypt	Friesian cattle	3625	-15 to 12
Calving interval:			
Amimo *et al*. (2006), Kenya	Ayrshire cattle	2757	-20 to 10
Ilatsia *et al*. (2007), Kenya	Sahiwal cattle	7211	-80 to 120
Ibrahim *et al*. (2009), Egypt	Holstein cattle	3656	-24 to 24
Osman *et al*. (2013), Egypt	Holstein-Friesian cattle	3460	-12 to 1.5
Ghiasi and Honarvar (2016), Iran	Iranian Holstein	72124	-7 to 3
Rahbar *et al* (2016), Iran	Holstein cattle	23402	0 to 7.2
El-Awady *et al*. (2017), Egypt	Friesian cattle	5728	-6 to 12
Zahed *et al*. (2020), Egypt	Friesian cattle	3635	-15 to 12
Caivio-Nasner *et al*. (2021), Colombia	Blanco Orejinegro cattle	729	-10 to 25
Kgari *et al*. (2023), South Africa	Holstein cattle	64464	-7.5 to 9

2.5.3 Reviewed predicted breeding values for semen traits

The estimates of predicted breeding values for semen traits as cited in buffalo and cattle literature were shown in **Table 8**. In buffalo, the ranges of breeding values were -0.45 to 3.32 *ml* for ejaculate volume, -4.3 % to 52 % for sperms motility, -5.8 to 8.1 % for live sperms and 799 to 1959 x 10^6 for sperms concentration (**El-Basuini, 2010; Kumar *et al*., 2023**). In cattle, the ranges of breeding values were -7.10 to 11.0 *ml* for ejaculate volume, -16.9 to

11.6 % for sperms motility and -336 to 428x10^6 for sperms concentration **(Olsen et al., 2020; Butler *et al*., 2021; Khattab *et al*., 2022**).

Table 8. Predicted breeding values (PBV) for semen traits as cited in buffalo and cattle literature

Reference and country of research	**Breed used**	**N**	**Ranges in PBV**
In buffalo:			
Ejaculate volume (*ml*):			
El-Basuini, (2010), Egypt	Egyptian buffalo	109	-0.45 to 0.45
Kumar *et al*. (2023), India	Indian buffalo	10975	2.19 to 3.32
Sperms motility (%):			
El-Basuini, (2010), Egypt	Egyptian buffalo	109	-4.3 to 5.6
Kumar *et al*. (2023), India	Indian buffalo	10975	46 to 52
Live sperms (%):			
El-Basuini (2010), Egypt	Egyptian buffalo	109	-5.8 to 8.1
Sperms concentration x10^6:			
Kumar *et al*. (2023), India	Indian buffalo	10975	799 to 1959
In cattle:			
Ejaculate volume (*ml*):			
Olsen *et al*. (2020), Norway	Norwegian Red cattle	14972	0.16
Butler *et al*. (2021), USA	American Angus cattle	44431	-7.1 to 11.0
Khattab *et al*. (2022) Egypt	Friesian cattle	14696	-0.7 to 0.8
Sperms motility (%):			
Olsen *et al*. (2020), Norway	Norwegian Red cattle	14972	0.8 to 2.4
Butler *et al*. (2021), USA	American Angus cattle	44418	-16.9 to 11.6
Khattab *et al*. (2022) Egypt	Friesian cattle	14696	-13.2 to 7.3
Sperms concentration x10^6:			
Butler *et al*. (2021), USA	American Angus cattle	44038	-336 to 428
Khattab *et al*. (2022), Egypt	Friesian cattle	14696	-259 to 239

2.5.4 Reviewed predicted breeding values for body weights

Reviewed estimates of predicted breeding values for body weights at birth and weaning as cited in buffalo and cattle literature were presented in **Table 9.** The breeding values in buffalo ranged from -4.3 to 3.4 kg for birth weight and -15.8 to 15.5 kg for weaning weight **(EL-Awady *et al*., 2005; Shahin et al., 2010; Sanghuayphrai *et al*., 2013; Gupta *et al*., 2015; Agudelo-Gómez *et al*., 2015; Salem *et al*., 2020; Elsayed *et al*., 2021)**. In cattle, the ranges in breeding values were -7.91 to 28.4 kg for BW, and -96 to

9.99 kg for WW **(Intaratham *et al*., 2008; Tawah *et al*., 1994; Koetz Júnior *et al*., 2017; Sanad and Gharib., 2017; Sharif-Islam and Bhuiyan., 2024).**

Table 9. Predicted breeding values for body weight at birth and weaning as cited in buffalo and cattle literature

Reference and country of research	Breed used	No. of calves	Ranges in PBV
In buffalo:			
Birth weight (kg):			
EL-Awady *et al*. (2005), Egypt	Egyptian buffalo	5405	-4.8 to 3.4
Shahin *et al*. (2010), Egypt	Egyptian buffalo	244	3.10
Elsayed *et al*. (2021), Egypt	Syrian buffalo	501	-0.01 to 0.03
Salem *et al*. (2020), Egypt	Egyptian buffalo	16370	-0.2 to 0.2
Weaning weight (kg):			
EL-Awady *et al*. (2005), Egypt	Egyptian buffalo	5405	-15.8 to15.5
Shahin *et al*. (2010), Egypt	Egyptian buffalo	244	1.20
Sanghuayphrai *et al*. (2013), Thailand	Swamp buffalo	4950	0.22 to 0.23
Gupta *et al*. (2015), India	Murrah buffalo	1055	0.45 to 0.36
Agudelo-Gómez *et al*. (2015), Colombia	Colombian buffalo	23947	-1.53 to 5.01
Elsayed *et al*. (2021), Egypt	Syrian buffalo	501	-0.02 to 0.09
Salem *et al*. (2020), Egypt	Egyptian buffalo	16370	-1.0 to 0.5
In cattle:			
Birth weight (kg):			
Intaratham *et al*. (2008), Thailand	Northeastern Thai indigenous cattle	1922	-0.60 to 0.14
Tawah *et al*. (1994), Cameroon	Wakwa and Ngaundere Gudali cattle	2211	-1 to 1.5
Koetz Júnior *et al*. (2017), Brazil	Nellore cattle	241471	1 to 6
Sanad and Gharib, (2017), Egypt	Friesian cattle	1691	-7.91 to 28.47
Sharif-Islam and Bhuiyan (2024), Bangladesh	Red Chittagong cattle	352	0.11 to 1.35
Weaning weight (kg):			
Intaratham *et al*. (2008), Thailand	Northeastern Thai indigenous cattle	1489	-2.96 to 1.21
Tawah *et al*. (1994), Cameroon	Wakwa and Ngaundere Gudali cattle	2172	-2 to 10
Sanad and Gharib, (2017), Egypt	Friesian cattle	1691	-96 to 9.99

2.6 Genetic and phenotypic trends

The genetic and phenotypic trends show the advancement or regression achieved with the followed breeding method **(Rege and Mosi, 1989; Njubi *et al.*, 1992; Ojango and Pollot, 2001)**. It is economically significant to quantify the genetic capacity of dairy animals, and the genetic trend indicates the improvement in genetic capacity **(Kunaka and Makuza, 2005)**. Improvements in breeding management and the environment can generally result in favourable phenotypic and genetic trends. Understanding the genetic improvement of a population enables the breeder to assess the gain of the breeding program, determine the gap between the selection goals and the gains made over time, and make the required corrections. Therefore, it is necessary to regularly assess the genetic and phenotypic parameters and trends in dairy cows in order to determine whether or not these trends and parameters are acceptable for each trait **(Amimo *et al.*, 2007)**.

2.6.1 Genetic and phenotypic trends for lactation traits

The estimates of genetic and phenotypic trends for milk, fat and protein yields as cited in buffalo literature (**Table 10)** showed irregular genetic and phenotypic trends in the Egyptian studies **(El-Bramony, 2014; Amin *et al.*, 2015)** or in the non-Egyptian studies **(Ahmad *et al.*, 2008; Pawar *et al.*, 2018)**. In development and evaluation of breeding programs in buffalo, the genetic parameters (e.g. heritability and predicted breeding values) need to be evaluated accurately in order to investigate the genetic, phenotypic and environmental trends. Several Egyptian studies have showed that the genetic and phenotypic trends for milk, fat and protein yields were favorable and showing an increase in both trends together **(e.g. Fooda *et al.*, 2010; El-Arian *et al.*, 2012; Ahmad *et al.*, 2017; Abo-Gamil *et al.*, 2017**), while few studies showed a decrease in the genetic and phenotypic trend together (**Khattab and Mourad, 1992; El-Bramon, 2014)**. Most of the non-Egyptian studies showed that genetic and phenotypic trends in milk, fat and protein yields in buffalo were increasing together **(e.g. Marques *et al.*, 1991; Sahana and Sadana, 1998; Kuralkar and Raheja, 2001; Ramos *et al.*, 2006; Ahmad, 2007; Poor *et al.*, 2012; Pawar *et al.*, 2018; Kour and**

Narang, 2021). In the other studies, the genetic and phenotypic trends were decreasing in milk yields and components **(Kuralkar and Raheja, 1997; Chakraborty and Dhaka, 2012),** while the genetic trend in milk yields was decreasing **(Sharma and Singh, 1992; Khan, 1998)** or was increasing **(Peeva and Krastanov, 2001; Catillo *et al.*, 2001; Seno *et al.*, 2010; Aspilcueta-Borquis *et al.*, 2015; Nazari *et al*., 2021)**.

Table 10. Estimates of genetic and phenotypic trends for milk yield and components as cited in buffalo literature

Reference and country of work	Breed used	No of records	Genetic trend	Phenotypic trend
Milk yield:				
Marques *et al*. (1991), Portugal	Murrah and Mediterranean	3991	Increased	Increased
Khattab and Mourad (1992), Egypt	Egyptian buffalo	1180	Decreased	Decreased
Sharma and Singh (1992), India	Murrah buffalo	478	Decreased	NE
Kuralkar and Raheja (1997), India	Murrah buffalo	2107	Decreased	Decreased
Sahana and Sadana (1998), India	Murrah buffalo	424	Increased	Increased
Khan (1998), Pakistan	Nili-Ravi buffalo	5341	Decreased	NE
Kuralkar and Raheja, (2001), India	Murrah buffalo	2017	Increased	Increased
Catillo *et al*. (2001), Italy	Italian buffalo	94028	Increased	NE
Ramos *et al*. (2006), Brazil	Murrah buffalo	3392	Increased	Increased
Ahmad (2007), Pakistan	Nili-Ravi buffalo	263	Increased	NE
Ahmad *et al*. (2008), Pakistan	Nili-Ravi buffalo	1524	Increased	Decreased
Mohamed *et al*. (2010), Egypt	Egyptian buffalo	3005	Increased	Increased

Table 10. Cont.

Reference and country of work	Breed used	No of records	Genetic trend	Phenotypic trend
Fooda *et al*. (2010), Egypt	Egyptian buffalo	3495	Increased	Increased
Seno *et al*. (2010), Brazil	Murrah buffalo	1578	Increased	NE
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	Increased	Increased
Chakraborty and Dhaka (2012), India	Murrah buffalo	1578	Decreased	Decreased
Poor *et al*. (2012), Iran	Iranian buffalo	4482	Increased	Increased
El-Bramony, (2014), Egypt	Egyptian buffalo	2066	Decreased	Decreased
Amin *et al*. (2015), Egypt	Egyptian buffalo	4971	Increased	Decreased
Aspilcueta-Borquis *et al*. (2015), Brazil	Murrah buffalo	5896	Increased	NE
Shalaby *et al*. (2016), Egypt	Egyptian buffalo	1776	Decreased	NE
Ahmad *et al*. (2017), Egypt	Egyptian buffalo	2763	Increased	Increased
Abo-Gamil *et al*. (2017), Egypt	Egyptian buffalo	1600	Increased	Increased
Pawar *et al*. (2018), India	Surti buffalo	1364	Decreased	Increased
EL-Hedainy *et al*. (2020), Egypt	Egyptian buffalo	1792	Increased	NE
Kour and Narang (2021), India	Murrah buffalo	675	Decreased	Decreased
Nazari *et al*. (2021), Iran	Italian buffalo	43818	Increased	NE
Fat yield:				
Marques *et al*. (1991), Portugal	Murrah and Mediterranean	3991	Increased	Increased
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	Increased	Increased
Poor *et al*. (2012), Iran	Iranian buffalo	4482	Increased	Increased
Amin *et al*. (2015), Egypt	Egyptian buffalo	4971	Increased	Decreased
Aspilcueta-Borquis *et al*. (2015), Brazil	Murrah buffalo	5896	Increased	NE
Kumar *et al*. (2016), India	Murrah buffalo	10981	Increased	Increased
Nazari *et al*. (2021), Iran	Italian buffalo	43818	Increased	NE
Protein yield:				
El-Arian *et al*. (2012), Egypt	Egyptian buffalo	3321	Increased	Increased
El-Bramony, (2014), Egypt	Egyptian buffalo	2066	Increased	Increased
Amin *et al*. (2015), Egypt	Egyptian buffalo	4971	Increased	Decreased
Aspilcueta-Borquis *et al*. (2015), Brazil	Murrah buffalo	5896	Increased	NE

NE = Not estimated.

2.6.2 Genetic and phenotypic trends for reproduction traits

The estimates of genetic and phenotypic trends for reproduction traits as cited in buffalo and cattle literature were showed in **Table 11.** In buffalo, the genetic and phenotypic trends in age at first calving and calving interval were increasing together **(Kour and Narang, 2021; Kour *et al*, 2021).** But, revisable trends were observed where the genetic trend was increasing and the phenotypic trend was decreasing or *vice versa* **(Catillo *et al*., 2001; Amin *et al*., 2021)**. **Bashir *et al*. (2009)** showed that favorable decreasing in genetic trends for age at first calving of Nili-Ravi buffalo in Pakistan, while **Gupta *et al*. (2015)** showed that unfavorable increase in genetic trend for age at first calving in Murrah buffalo**.** In Egyptian buffalo**, Shalaby *et al*. (2016)** reported that the phenotypic and genetic trends for calving interval and days open were decreased, while **Amin *et al*. (2021)** found that the genetic trends were increasing in these traits. In Murrah buffalo, **Kour and Narang (2021)** and **Kour *et al*. (2021)** reported that the phenotypic and genetic trends for age at first calving and first calving interval were increased. In cattle, the Egyptian studies reported that the genetic and phenotypic trends for age at first calving, days open and calving interval were favorable and showing an increase in both trends together **(*e.g.* El-Awady *et al*., 2017; Zahed *et al*., 2020**). Also, most of the non-Egyptian studies showed that the genetic and phenotypic trends for age at first calving and calving interval were increasing together **(*e.g.* Amimo *et al*., 2006; Ilatsia *et al*., 2007; Orenge *et al*., 2009; Kgari *et al*., 2023).** In the other studies, both genetic and phenotypic trends were decreasing in age at first calving and calving interval **(Rahbar *et al*., 2016),** while the trend were decreasing **(Chaudhary *et al*., 1995; Ghiasi and Honarvar, 2016; Rahbar *et al*., 2016; Caivio-Nasner *et al*., 2021; Kgari *et al*., 2023)** or was increasing **(Ghiasi and Honarvar, 2016; Rahbar *et al*., 2016; Kgari *et al*., 2023)**.

Table 11. Estimates of genetic and phenotypic trends for reproduction traits as cited in buffalo and cattle literature

Reference and country of work	Breed used	No of records	Genetic trend	Phenotypic trend
In buffalo:				
Age at first calving:				
Catillo *et al*. (2001), Italy	Italian buffalo	94028	Decreased	Increased
Bashir *et al*. (2009), Pakistan	Nili-Ravi buffalo	2169	Decreased	Increased
Gupta *et al*. (2015), India	Murrah buffalo	725	Increased	NE
Kour and Narang (2021), India	Murrah buffalo	659	Increased	Increased
Kour *et al*. (2021), India	Murrah buffalo	659	Increased	Increased
Amin *et al*. (2021), Egypt	Egyptian buffalo	38906	Increased	Decreased
Days open:				
Shalaby *et al*. (2016), Egypt	Egyptian buffalo	1779	Decreased	Decreased
Amin *et al*. (2021), Egypt	Egyptian buffalo	38906	Increased	Decreased
Calving interval:				
Catillo *et al*. (2001), Italy	Italian buffalo	94028	Decreased	NE
El-Bramony, (2014), Egypt	Egyptian buffalo	2066	Decreased	Decreased
Shalaby *et al*. (2016), Egypt	Egyptian buffalo	1779	Decreased	Decreased
Kour and Narang (2021), India	Murrah buffalo	659	Increased	Increased
Kour *et al*. (2021), India	Murrah buffalo	659	Increased	Increased
Amin *et al*. (2021), Egypt	Egyptian buffalo	38906	Increased	NE
In cattle:				
Age at first calving:				
Chaudhary *et al*. (1995), India	Kankrej cattle	1344	Decreased	Increased
Amimo *et al*. (2006), Kenya	Ayrshire cattle	2757	Increased	Increased
Ibrahim *et al*. (2009), Egypt	Holstein cattle	3656	Increased	Decreased
Osman *et al*. (2013), Egypt	Holstein-Friesian cattle	3460	Increased	NE
Zahed *et al*. (2020), Egypt	Friesian cattle	3625	Increased	Increased
Kgari *et al*. (2023), South Africa	Holstein cattle	64464	Increased	Increased
Days open:				
Osman *et al*. (2013), Egypt	Holstein-Friesian cattle	3460	Increased	NE
Ghiasi and Honarvar (2016), Iran	Iranian Holstein	72124	Increased	Decreased
Awady *et al*. (2017), Egypt	Friesian cattle	5728	Increased	Increased
Rahbar *et al* (2016), Iran	Holstein cattle	23402	Increased	Decreased
Zahed *et al*. (2020), Egypt	Friesian cattle	3625	Increased	Increased

Table 11. Cont.

Reference and country of work	Breed used	No of records	Genetic trend	Phenotypic trend
Calving interval:				
Amimo *et al*. (2006), Kenya	Ayrshire cattle	2757	Increased	Increased
Ilatsia *et al*. (2007), Kenya	Sahiwal cattle	7211	Increased	Increased
Orenge *et al*. (2009), Kenya	Charolais and Hereford beef cattle	2117	Increased	NE
Ibrahim *et al*. (2009), Egypt	Holstein cattle	3656	Increased	Increased
Osman *et al*. (2013), Egypt	Holstein-Friesian cattle	3460	Increased	NE
Ghiasi an Honarvar (2016), Iran	Iranian Holstein	72124	Increased	Decreased
Rahbar *et al* (2016), Iran	Holstein cattle	23402	Decreased	Decreased
Awady *et al*. (2017), Egypt	Friesian cattle	5728	Increased	Increased
Zahed *et al*. (2020), Egypt	Friesian cattle	3635	Increased	Increased
Caivio-Nasner *et al*. (2021), Colombia	Blanco Orejinegro cattle	729	Decreased	Increased
Kgari *et al*. (2023), South Africa	Holstein cattle	64464	Increased	Decreased

NE = Not estimated.

2.6.3 Genetic and phenotypic trends for semen traits

The reviewed studies in **Table 12** showed that the genetic and phenotypic trends for semen traits were favorable and showing an increase in both trends in different breeds of buffalo and cattle **(*e.g.* Olsen *et al*., 2020; Kumar *et al*., 2023)**. **Olsen *et al*. (2020)** found that the genetic trends in ejaculate volume, sperms motility and sperms concentration were increased in Norwegian Red cattle. **Kumar *et al*. (2023)** showed that genetic and phenotypic trends were positive and showing favorable increase in ejaculate volume and sperms motility in India buffalo.

Table 12. Estimates of genetic and phenotypic trends for semen traits as cited in buffalo and cattle literature

Reference and country of work	Breed used	No of records	Genetic trend	Phenotypic trend
In buffalo:				
Ejaculate volume:				
Kumar *et al*. (2023), India	India buffalo	10975	Increased	Increased
Sperms motility:				
Kumar *et al*. (2023), India	India buffalo	10975	Increased	Increased
Sperms concentration:				
Kumar *et al*. (2023), India	India buffalo	10975	Decreased	Decreased
In cattle:				
Ejaculate volume:				
Olsen *et al*. (2020), Norway	Norwegian red cattle	14972	Increased	NE
Sperms motility:				
Olsen *et al*. (2020), Norway	Norwegian red cattle	14972	Increased	NE
Sperms concentration:				
Olsen *et al*. (2020), Norway	Norwegian red cattle	14972	Increased	NE

NE = Not estimated.

2.6.4 Genetic and phenotypic trends for body weight

Most of the reviewed studies in **Table 13** showed that the genetic and phenotypic trends for birth and weaning weight were favorable and showing an increase in both trends in different breeds of buffaloes **(e.g. Malhado *et al*., 2007; Gupta *et al*., 2015; Elsayed *et al*., 2021; Salem *et al*., 2020)**. **Malhado *et al*., (2007)** showed that genetic and phenotypic trends were positive and showing favorable increase in body weights in Brazilian buffalo. **El-Bramony, (2014)** stated that the genetic and phenotypic trends for body weights were favorable and showing an increase in both trends**. Gupta *et al*. (2015)** showed that genetic trend was increasing in weaning weight of Murrah buffalo. **Elsayed *et al*. (2021)** found that the phenotypic and genetic changes in birth and weaning weights were increased in Syrian buffalo.

Table 13. Estimates of genetic and phenotypic trends for body weights as cited in buffalo and cattle literature

Reference and country of work	Breed used	No of records	Genetic trend	Phenotypic trend
In buffalo:				
Birth weight:				
Malhado *et al*. (2007), Brazil	Brazilian buffalo	6992	Increased	Increased
Elsayed *et al*. (2021), Egypt	Syrian buffalo	501	Increased	Increased
Salem *et al* (2020), Egypt	Egyptian buffalo	16370	Increased	Increased
Weaning weight:				
Gupta *et al*. (2015), India	Murrah buffalo	1055	Increased	NE
Elsayed *et al*. (2021), Egypt	Syrian buffalo	501	Increased	Increased
Salem *et al* (2020), Egypt	Egyptian buffalo	16370	Increased	Increased
In cattle:				
Birth weight:				
Tawah *et al*. (1994), Cameroon	Wakwa and Ngaundere Gudali cattle	2211	Increased	Increased
Intaratham *et al*. (2008), Thailand	Northeastern Thai indigenous cattle	1922	Increased	Increased
Sanad and Gharib, (2017), Egypt	Friesian cattle	1691	Increased	NE
Koetz Júnior *et al*. (2017), Brazil	Nellore cattle	241471	Increased	Increased
Ramírez Toro *et al*. (2020), Colombia	Blanco Orejinegro cattle	7304	Increased	NE
Sharif-Islam and Bhuiyan, (2024), Bangladesh	Red Chittagong cattle	352	Increased	Increased
Weaning weight:				
Tawah *et al*. (1994), Cameroon	Wakwa and Ngaundere Gudali cattle	2211	Increased	Increased
Intaratham *et al*. (2008), Thailand	Northeastern Thai indigenous cattle	1489	Increased	Increased
Araújo *et al*. (2010), Brazil	Angus-Nellore cattle	39676	Increased	Decreased
Orenge *et al*. (2009), Kenya	Charolais and Hereford beef	1869	Decreased	NE
Sanad and Gharib, (2017), Egypt	Friesian cattle	1691	Increased	NE
Ramírez Toro *et al*. (2020), Colombia	Blanco Orejinegro cattle	1281	Increased	NE
Sharif-Islam and Bhuiyan, (2024), Bangladesh	Red Chittagong cattle	245	Increased	Increased

NE = Not estimated.

In several reviewed studies, the genetic and phenotypic trends for birth and weaning weights in cattle were favorable and showing an increase in both trends together **(*e.g.* Tawah *et al*., 1994; Intaratham *et al*., 2008); Sanad and Gharib, 2017; Koetz Júnior *et al*., 2017; Ramírez Toro *et al*., 2020; Sharif-Islam and Bhuiyan, 2024)**. In cattle, the Egyptian studies reported that the genetic and phenotypic trends for birth and weaning weights were favorable and showing an increase in both trends together **(Sanad and Gharib, 2017**). Also, most of the non-Egyptian studies showed that the genetic and phenotypic trends for birth weight and weaning weight were increasing together **(e.g. Tawah *et al*., 1994; Intaratham *et al*., 2008; Koetz Júnior *et al*., 2017; Ramírez Toro *et al*., 2020; Sharif-Islam and Bhuiyan, 2024).** In the other studies, both genetic trends were decreasing in weaning weight **(Orenge *et al*., 2009),** while the phenotypic trends were decreasing **(Araújo *et al*., 2010)**.

2.7 PCR-RFLP and *SNP* techniques in DNA polymorphism in buffalo

In modern molecular studies of buffalo populations, *SNP*s and PCR-RFLP are widely advised to be utilized in genetic improvement programs in buffalo. In the last decade, these studies could provide great markers for the investigation of polymorphism of candidate genes to be used in marker assisted selection (MAS) or gene-assisted selection programs to improve the selection response of productive and reproduction traits in buffalo **(Pauciullo *et al*., 2012a,b; Gil *et al*., 2013; Zetouni *et al*., 2013, 2014; Araújo *et al*., 2015; Jamuna *et al*., 2016; Machado *et al*., 2016a; Deng *et al*., 2016; Freitas *et al*., 2016; Nadeem and Maryam, 2016; Konca and Akyüz., 2017; Kumar *et al*., 2017; Mavi *et al*., 2017; Ahmadzadeh *et al*., 2019; Al-Shawa *et al*., 2019; Silva *et al*., 2021**; **El Nagar *et al*, 2023)**. The PCR-RFLP technique is a traditional molecular method for identifying the genotypes structure of the buffalo populations and identifying the genetic polymorphisms caused by the presence of significant genes **(Yang *et al*., 2013)**. The two primary stages to be used in PCR-RFLP approach are the amplification of DNA using a normal PCR and the digestion of the PCR

product using restriction enzymes. The first step in PCR-RFLP is always to build the best primer combination and the second step is to discover the restriction enzymes to detect the *SNP*s in the PCR-amplified output. The *SNP* type is easily identified by using gel electrophoresis for separating and generating smaller sizes of DNA fragments by the endonuclease digestion.

Maudet *et al.* (2002) and **Erhardt and Weimann (2007)** stated that the most molecular markers are microsatellite markers, STR (Short Tandem Repeats) and *SNP* (Single Nucleated Polymorphism). **Seidel (2009)** noted that the genomic selection using the *SNP* markers is a powerful tool because: 1) *SNP* can be detected by a range of methods such as PCR-RFLP, 2) the DNA chips used in SNP technology can be used for large-scale screening of numerous samples in minimal time, and 3) SNP is the most recent contribution in the study of DNA sequence variation. However, recent advances in DNA sequencing, computer software and bioinformatics have been facilitated the identification of SNPs as molecular markers in buffalo **(Abo Al-Ela *et al*., 2014; Kathiravan *et al*., 2019; Erdoğan *et al.*, 2021; El-Magd *et al.*, 2021)**.

Mapping quantitative trait loci (QTL) was the perfect approach to identify genes associated with complex traits in Genome Wide Association Studies (GWAS) using the molecular markers. In the last decade, GWAS using the technique of single nucleotide polymorphism (SNP) has been used. Accordingly, GWAS has gained significant road in the recent years, enabling the rapid advancements in genotyping and molecular technologies in buffalo **(De Camargo *et al*., 2015; Li *et al*., 2018a; Li *et al*., 2018b; Shao *et al*., 2021; Rehman *et al*., 2022; EL Nagar *et al*., 2023)**. This method facilitates the identification of precisely QTL by examining the associations between genetic markers and the phenotypic values of the individuals and therefore can leading to succession in breeding programs in buffalo (**El-Halawany *et al*, 2017; Abdel-Shafy *et al*, 2020**). So far, GWAS using this buffalo SNP chip (Axiom Buffalo Genotyping 90k by Affymetrix) has involved in Italian Mediterranean buffalo **(Iamartino *et al*., 2013),** in Brazilian buffalo (**De Camargo *et al*., 2015)** and in Egyptian buffalo **(El-Halawany *et al*., 2017)**.

2.8 Candidate genes and their molecular associations with lactation traits in buffalo:

The candidate genes associated with lactation traits cited in the molecular studies in buffalo are summarized in **Tables 14** and **15**. According to the available molecular facts mentioned in these tables along with buffalo GWAS conducted for lactation traits**, (Iamartino *et al*., 2013; De Camargo *et al*., 2015; El-Halawany *et al*., 2017; Abdel-Shafy *et al*, 2020; Liu *et al*., 2020; Rehman *et al*., 2022; Lázaro *et al*., 2024)**, we can summarize the candidate genes controlling lactation traits significantly in buffalo as follows:

On chromosome 1, Melatonin receptor 1A gene (*MTRN1A)* was detected in the Brazilian buffalo (**Zetouni *et al*., 2014; Machado *et al*., 2016a; Machado *et al*., 2016b)** and Pituitary specific transcription factor-1 gene (*Pit1*) were detected in the Egyptian buffalo (**Othman *et al*., 2012**), Murrah buffalo (**Mavi *et al*., 2017)** and Khuzestan water buffalo **(Ahmadzadeh *et al*., 2019**). These genes are associated significantly with milk, fat and protein production. On chromosome 2, Prolactin gene **(***PRL*) was associated with milk, fat and protein yields in Indian buffalo **(Ladani *et al*., 2003; Madnalwar *et al*, 2010),** Nili-Ravi buffalo **(Nadeem and Maryam, 2016)**, Anatolian water buffalo **(Konca and Akyüz., 2017)**, Murrah buffalo (**Mavi *et al*., 2017),** Mediterranean Italian buffalo **(Li *et al*., 2017)** and Egyptian buffalo **(Othman *et al*., 2012; El-Magd *et al*., 2015; El-Komy *et al*., 2020; Abd El Fattah *et al*., 2023**). On chromosome 3, Adrenoceptor alpha 1A gene (*ADRAIA*) in Brazilian buffalo **(Araújo *et al*., 2015),** Fatty acid synthase gene (*FASN*) in Murrah buffalo **(Kumar *et al*., 2017**) and Growth hormone gene (*GH*) in Egyptian buffalo (**Othman *et al*, 2012; El-Komy *et al*, 2020)** and in Khuzestan water buffalo **(Ahmadzadeh *et al*, 2019),** all these genes are associated with milk yield and/or milk compositions. On chromosome 4, Insulin like growth factor 1 gene (*IGF-I*) in Murrah buffalo **(Freitas *et al*., 2016**) and Alpha-2-macroglobulin gene *(A2M*) in Meshing, Surti and Jaffarabadi buffalo (**Shadma *et al*., 2009),** Asian water buffalo **(Ramesha *et al*., 2015)** and the Egyptian buffalo **(El-Komy *et al*., 2020; Ali *et al*., 2023**) and Alpha-lactalbumin gene (*ALA*) in Nili Ravi buffalo

(Sihag *et al*., 2023) are associated with milk yield and constituents. On chromosome 6, Casein alpha S2 gene (*CSNS2*) was associated with milk yield, fat, protein, casein, solids not fat and total solids in Bhadawari, Murrah, Mehsana and Surti buffalo **(Misra *et al*., 2008**). On chromosome 7, Kappa-casein gene (*CSN3*) is associated with milk yield, fat, protein, lactose and total solids in Murrah buffalo (**Otaviano *et al*., 2005),** Khuzestan water buffalo **(Ahmadzadeh *et al*., 2019)** and Egyptian buffalo **(Othman *et al*., 2012; Al-Shawa *et al*., 2019; El-Komy *et al*., 2020)**, Alpha-S2-casein-like gene (*CSN1S2*) is associated with milk fatty acid and composition in Italian river buffalo (**Cosenza *et al*., 2021),** and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha gene (*PPARGC1A*) is associated with milk yield in Italian Mediterranean buffalo (**Hosseini *et al*., 2021**) and Murrah and Bhadawari buffalo **(Sihag *et al*., 2023)**. On chromosome 8, Insulin-like growth factor binding protein-3 gene (*IGF*BP-*3*) is associated with milk yield in Asian water buffalo **(Ramesha *et al*., 2015)**, Leptin gene (*LEP*) is associated with fat and protein yields in Mehsana, Marathwada, Chilika, Jaffarabadi, Murrah, Nili-Ravi, Pandharpuri and Toda buffalo **(Tanpure *et al*., 2012),** Egyptian buffalo **(Abdo *et al*., 2014; Mahrous *et al*. 2020; Ali *et al*., 2023),** Murrah buffalo **(Jamuna *et al*., 2016),** Anatolian Water buffalo **(Kaplan, 2018)** and Murrah and Mediterranean buffalo **(Silva *et al*., 2021)**. The genes of Oxytocin/neurophysin I (OXT) and Growth Hormone Releasing Hormone (GHRH) located on chromosome 14 are associated with milk production **(Pauciullo *et al*., 2012a; Araújo *et al*., 2015**; **Ahmadzadeh *et al*., 2019).** Diacylglycerol O-acyltransferase 1 (DGAT1) located on chromosome 15 is associated with milk production in Chinese buffalo (**Yuan *et al*., 2007),** Anatolian buffalo **(Özdil and Ilhan, 2012),** Mazandaran, Khuzestan, Guilan, Azerbayjan and Kermanshah buffalo **(Heydarian *et al*., 2014),** Murrah buffalo (**Freitas *et al*., 2016; Sulabh *et al*., 2018; Krovvidi *et al*., 2021),** Murrah and Mediterranean buffaloes **(Silva *et al*., 2016),** Mehsana buffalo (**Parikh *et al*., 2016),** Riverine and Swamp buffaloes **(Li *et al*., 2018c),** Iraqi buffalo **(Kadhim and Ibrahim, 2019),** Egyptian buffalo **(El-Komy *et al*., 2020; Sihag et al., 2024)** and Brazilian buffalo **(Khan *et al*., 2021)**. A set of

genes located on chromosome 19 are associated with milk yield such as Casein alpha s1 CSNS1 in Bhadawari, Murrah, Mehsana and Surti buffalo (**Misra *et al*., 2008**), Growth hormone receptor gene (*GHR*) in Egyptian buffalo (**Othman *et al*., 2012),** Asian water buffalo **(Ramesha *et al*., 2015)** and Anatolian buffalo **(Erdoğan *et al*., 2021)** and Prolactin-like receptor gene in Murrah buffalo (**Al-Kal *et al*., 2018),** Jaffarabadi and Surti buffalo (**Devkatte *et al*., 2021)** and Egyptian buffalo **(El-Komy *et al*., 2020; El-Magd *et al*., 2021; Abd El Fattah *et al*., 2023)**.

Table 14. The Candidate genes molecularly associated with milk production and composition traits as cited in Egyptian buffalo studies

CN	Candidate gene	Reference
1	Pituitary-specific transcription factor-1 (*Pit1*)	Othman *et al*. (2012)
2	Prolactin (*PRL*)	Othman *et al*., 2012; El-Magd *et al*., 2015; El-Komy *et al*., 2020; Abd El Fattah *et al*., 2023
3	Growth hormone (*GH*)	Othman *et al*. (2012), El-Komy *et al*. (2020)
4	Insulin like growth factor 1 (*IGF*-1)	El-Komy *et al*. (2020), Ali *et al*. (2023)
7	Kappa-Casein gene (*CSN*)	Othman *et al*. (2012), Al-Shawa *et al*. (2019), El-Komy *et al*. (2020)
8	Leptin gene (*LEP*)	Abdo *et al*. (2014), Mahrous *et al*. (2020), Ali *et al*. (2023)
15	Diacylglycerol O-acyltransferase 1 gene (*DGAT1*)	El-Komy *et al*. (2020)
19	Growth hormone receptor (*GHR*) Prolactin-like receptor gene (*PRLR*)	Othman *et al*. (2012), El-Komy *et al*. (2020)
20	Insulin like growth factor 1 receptor (*IGF-1R*)	Abd El Fattah *et al*. (2023), Ali *et al*. (2023)

CN= Chromosome number

Table 15. The candidate genes listed according to their chromosomal number, molecularly associated with lactation traits as cited in non-Egyptian buffalo studies

CN	Candidate gene	Buffalo breed	Reference and country of work
1	Melatonin receptor 1A (*MTRN1A*)	Brazilian	Zetouni *et al*. (2014) , Brazil
		Amazon	Machado *et al*. (2016b), Brazil
		Brazilian	Machado *et al*. (2016a), Brazil
	Pituitary-specific transcription factor-1 (*Pit1*)	Murrah	Mavi *et al*. (2017), India
		Khuzestan	Ahmadzadeh *et al*. (2019), Iran
2	Prolactin (*PRL*)	Indian	Ladani *et al*. (2003), Madnalwar *et al*, 2010, India
		Nili-Ravi	Nadeem and Maryam (2016), Pakistan
		Italian Mediterranean	Li *et al*. (2017), China
		Murrah	Mavi *et al*. (2017), India
		Anatolian water	Konca and Akyüz. (2017), Turkey
		Anatolian	Özşensoy (2018), Turkey
3	Adrenoceptor alpha 1A (*ADRAIA*)	Brazilian	Araújo *et al*. (2015), Brazil
	Fatty acid synthase (*FASN*)	Murrah	Kumar *et al*. (2017), India
	Growth hormone (*GH*)	Khuzestan	Ahmadzadeh *et al*. (2019), Iran
4	Alpha-2-macroglobulin *(A2M*)	Murrah	Freitas *et al*. (2016), Brazil
	Insulin like growth factor 1 (*IGF-I*)	Meshing, Surti, Jaffarabadi	Shadma *et al*. (2009), India
		Asian water	Ramesha *et al*. (2015), India
	Alpha-lactalbumin (*ALA*)	Ravi	Sihag *et al*. (2023), India
6	Casein alpha S2 (*CSNS2*)	Bhadawari , Murrah, Mehsana , Surti	Misra *et al*. (2008), India
7	Kappa-casein N3 (*CSN3*)	Murrah	Otaviano *et al*. (2005) , Brazil
		Khuzestan water	Ahmadzadeh *et al*. (2019), Iran
	Alpha-S2-casein (*CSN1S2*)	Italian River	Cosenza *et al*. (2021), Italy
	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*)	Italian Mediterranean	Hosseini *et al*. (2021), China
		Murrah and Bhadawari	Sihag *et al*. (2023), India

Table 15. Cont.

CN	Candidate gene	Buffalo breed	Reference and country of work
8	Insulin-like growth factor binding protein-3 (*IGF*BP-*3*)	Asian water	Ramesha *et al*. (2015), India
	Leptin (*LEP*)	Mehsana, Marathwada, Chilika, Jaffarabadi, Murrah, Nili-Ravi, Pandharpuri, Toda	Tanpure *et al*. (2012), India
		Brazilian	Zetouni *et al*. (2013), Brazil
		Murrah	Jamuna *et al*. (2016), India
		Anatolian Water	Kaplan, (2018), Turkey
		Murrah, Mediterranean	Silva *et al*. (2021), Brazil
		Egyptian	Ali *et al*. (2023), Egypt
14	Oxytocin/neurophysin I (*OXT*)	Mediterranean Italiana	Pauciullo *et al*. (2012a), Italy
		Brazilian	Araújo *et al*. (2015), Brazil
	Growth hormone releasing hormone (*GHRH*)	Khuzestan	Ahmadzadeh *et al*. (2019), Iran
15	Diacylglycerol O-acyltransferase 1 (*DGAT1*)	Chinese	Yuan *et al*. (2007), China
		Anatolian	Özdil and Ilhan (2012), Turkey
		Mazandaran, Khuzestan, Guilan, Azerbayjan and Kermanshah	Heydarian *et al*. (2014), Iran
		Murrah	Freitas *et al*. (2016), Brazil
		Murrah and Mediterranean	Silva *et al*. (2016), Brazil
		Mehsana	Parikh *et al*. (2016), India
		Riverine and Swamp	Li *et al*. (2018)c, China
		Murrah	Sulabh *et al*. (2018), India
		Iraqi	Kadhim and Ibrahim (2019), Iraq
		Murrah	Krovvidi *et al*. (2021), India
		Brazilian	Khan *et al*. (2021), Brazil
19	Casein alpha S1 (*CSNS1*)	Bhadawari , Murrah, Mehsana, Surti	Misra *et al*. (2008), India
	Growth hormone receptor (*GHR*)	Asian water	Ramesha *et al*. (2015), India
		Anatolian	Erdoğan *et al*. (2021), Turkey
	Prolactin-like receptor (*PRLR*)	Murrah	Al-Kal *et al*. (2018), China
		Jaffarabadi and Surti	Devkatte *et al*. (2021), India

Table 15. Cont.

CN	Candidate gene	Buffalo breed	Reference and country of work
21	Oxytocin receptor (*OXTR*)	Italian Mediterranean	Cosenza *et al*. (2017), Italy
	Ghrelin (*GHRL*)	Brazilian	Gil *et al*. (2013), Brazil
	Lactoferrin (*LTF*)	Murrah	Singh *et al*. (2020), India
22	Melanocortin 4 receptor (*MC4R*)	Chinese	Deng *et al*. (2016), China
		Murrah	Singh *et al*. (2020), India
23	Ornithine aminotransferase (*OAT*)	Chinese	Ma *et al*. (2021), China
	Stearoyl-CoA desaturase (*SCD*)	Murrah	Pauciullo *et al*. (2012b), Italy
		Murrah, Mediterranean	Silva *et al*. (2021), Brazil

CN= Chromosome number

On chromosome 20, Insulin like growth factor 1 receptor (*IGF-1R)* is associated with several lactation traits in Egyptian buffalo **(Ali *et al*., 2023)**. On chromosome 21, Oxytocin receptor gene (*OXTR*) is associated with milk yield and fatty acids in Italian Mediterranean River buffalo **(Cosenza *et al*., 2017)**, while Ghrelin gene (*GHRL*) is associated with milk, fat, and protein yields in Brazilian Water buffalo **(Gil *et al*., 2013**) and Lactoferrin gene (*LTF*) in Murrah buffalo **(Singh *et al*., 2020)**. On chromosome 22, Melanocortin 4 receptor gene (*MC4R*) is associated with milk production in Chinese Water buffalo (**Deng *et al*., 2016)** and Murrah buffalo **(Singh *et al*., 2020)**. On chromosome 23, Stearoyl-CoA desaturase gene (*SCD*) is associated with milk yield in Murrah and Mediterranean buffalo **(Pauciullo *et al*., 2012b; Silva *et al*., 2021)** and Ornithine aminotransferase gene (*OAT*) in Chinese buffalo **(Ma *et al*., 2021)**.

2.9 Candidate genes and their molecular associations with reproduction traits in buffalo

The available studies showing significant associations of candidate genes with reproduction traits in buffalo are given in **Table 16**. These genes along with those listed in GWAS literature detected on chromosome number 1, 2, 12, 19, and 22 are molecularly associated reproduction traits in buffalo such as stillbirth, calving ease, gestation length, postpartum interval to pregnancy, calving interval, and age at first calving in buffalo (**De Camargo *et al*., 2015; Li *et al*., 2018a; Li *et al*., 2018b; Shao *et al*., 2021; Abd El Fattah *et al*., 2023)**. Melatonin receptor 1A gene (*MTRN1A*) detected on chromosome 1 is associated with age at first calving and calving interval in Brazilian buffalo (**Zetouni *et al*., 2014)**. Prolactin gene (*PRL*) detected on chromosome 2 is associated with stillbirth, calving ease, gestation length, postpartum interval to pregnancy, calving interval, and age at first calving in Egyptian buffalo **(Abd El Fattah *et al*., 2023)**. On chromosome 12, several candidate genes, like Follicle-stimulating hormone receptor gene (*FSHR*) is associated with ovarian status, fertility traits, age at first calving, calving interval, days open, stillbirth and calving ease in Egyptian buffalo (**Othman and Abdel-samad, 2013; Sosa *et al*., 2015; Shafik *et al*., 2017; El-Debaky *et al*., 2020; Ramadan *et al*., 2020; Sallam *et al*., 2022; Wagdy *et al*., 2023)** and in Murrah buffalo **(Kathiravan *et al*., 2019),** Luteinizing hormone receptor gene (*LHR*) is associated with ovarian status and fertility traits in Egyptian buffalo **(Othman and Abdel-samad, 2013; Sosa *et al*., 2016)** and Estrogen receptor-α gene (*ERα*) in Egyptian buffalo (**Othman and Abdel-samad, 2013)**. Prolactin-like receptor gene (*PRLR*) detected on chromosome 19 is associated with stillbirth, calving ease, gestation length, postpartum interval to pregnancy, calving interval, and age at first calving in Egyptian buffalo **(Abd El Fattah *et al*., 2023)**, while Lactoferrin gene (*LTF*) detected on chromosome 22 is associated with fertility traits in Egyptian buffalo (**El-Debaky *et al*., 2020)**.

Table 16. The list of candidate genes according to their chromosomal number, molecularly associated with reproduction traits as cited in buffalo literature

CN	Candidate gene	Buffalo breed	Reference and country of work
1	Melatonin receptor 1A (*MTRN1A*)	Brazilian	Zetouni *et al*. (2014) , Brazil
2	Prolactin (*PRL*)	Egyptian	Abd El Fattah *et al*. (2023), Egypt
		Indian	Madnalwar *et al*, 2010, India
12	Follicle-stimulating hormone receptor (*FSHR*)	Egyptian	Othman and Abdel-samad (2013), Egypt
		Egyptian	Sosa *et al*. (2015), Egypt
		Egyptian	Shafik *et al*. (2017), Egypt
		Murrah	Kathiravan *et al*. (2019), India
		Egyptian	Ramadan *et al*. (2020), Egypt
		Egyptian	Fouda *et al*. (2021), Egypt
		Egyptian	Sallam *et al*. (2022), Egypt
		Egyptian	Wagdy *et al*. (2023), Egypt
	Luteinizing hormone receptor (*LHR*)	Egyptian	Othman and Abdel-samad (2013), Egypt
		Egyptian	Sosa *et al*. (2016), Egypt
	Estrogen receptor-α (*ERα*)	Egyptian	Othman and Abdel-samad (2013), Egypt
19	Prolactin-like receptor (*PRLR*)	Egyptian	Abd El Fattah *et al*. (2023), Egypt
22	Lactoferrin (*LTF*)	Egyptian	El-Debaky *et al*. (2020), Egypt

CN= Chromosome number

2.10 Candidate genes and their molecular associations with semen traits in buffalo

In the last decade, few studies have shown significant associations of candidate genes with semen traits in buffalo **(Table 17)**. According to the available molecular results mentioned in **table 17** along with those available in GWAS (**Rehman *et al*., 2022; EL Nagar *et al*., 2023**), semen traits are significantly controlled by the following candidate genes in buffalo. On chromosome 1, Pituitary-specific transcription factor gene (*PIT-1*) and Sperm associated antigen 11B gene (*SPAG11B*) are associated with ejaculate volume, individual sperm motility, live sperms and chromatin sperm damage in Egyptian buffalo **(Hasanain *et al*., 2016)** and Murrah buffalo **(Deshmukh *et al*., 2021)**. On chromosome 2, Transition nuclear protein-1 gene (*TNP-1*) is

associated with immature sperms in Murrah buffalo **(Panigrahi and Yadav, 2010)**, Capping actin protein Z-line beta subunit gene (*CAPZB*) is associated with sperm motility in Murrah buffalo **(Xiong *et al.*, 2018**), Heat shock protein 70 gene (*HSP70*) and Prolactin gene (*PRL*) are associated with several semen quality traits in Egyptian buffalo **(Gafer *et al*. 2015; Hasanain *et al.*, 2017)**. For genes located on chromosome 3, Growth hormone gene (*GH*) is associated with semen volume, individual sperm motility, sperms concentration, abnormalities and live sperms in Egyptian buffalo **(Darwish *et al*. 2016)**, while prohibitin gene (*PHB*) is associated with sperms motility in Murrah buffalo **(Xiong *et al*. 2018**). Aquaporin 7 gene (*AQP7*) located on chromosome 3 in Murrah buffalo **(Kumari *et al*. 2018; Wang *et al*. 2020**), Leptin gene (*LEP*) located on chromosome 8 **(Dilbar *et al*. 2019**), Luteinizing hormone receptor gene (*LHR*) located on chromosome 12 **(Reen *et al*. 2018)**, and Inhibit alpha (*INHA*) located on chromosome 17 **(Chandra *et al*. 2020**) are associated with sperms quality. On chromosome 6, Tektin-2 gene (*TEKT2)* is associated with sperms motility in Murrah buffalo (**Xiong *et al,* 2018)**. On chromosome 7, Secreted phosphoprotein 1 gene (*SPP1*) is associated with semen production in Brazilian water buffalo (**Rolim Filho *et al.*, 2013)** and Gonadotropin releasing hormone receptor gene (*GnRHR*) is associated with semen volume, sperms concentration, sperms motility, live sperms and sperms abnormality in Chinese water buffalo **(Wang *et al.*, 2017)** and Egyptian buffalo **(Mahmoud *et al.*, 2021)**. On chromosome 18, Luteinizing hormone beta gene (*LHβ*) is associated with sperms concentration and mass sperms motility percent in Murrah buffalo **(Reen *et al,* 2018)**. On sex chromosome X (chromosome 25), four novel genes (Melanoma-associated antigen D2 gene, Cancer/testis antigen 47A-like gene, Actin-related protein T1 gene, Sodium/hydrogen exchanger 2-like gene) were detected in Egyptian buffalo **(EL Nagar *et al.*, 2023**).

Table 17. The list of candidate genes molecularly associated with semen traits as cited in buffalo literature

CN	Candidate gene	Buffalo breed	Reference and country of work
1	Pituitary-specific transcription factor 1 (PIT-1)	Egyptian	Hasanain *et al*. (2016), Egypt
	Sperm associated antigen 11B (SPAG11B)	Murrah	Deshmukh *et al*. (2021), India
2	Transition nuclear protein-1 (*TNP-1*)	Murrah	Panigrahi and Yadav (2010), India
	Capping actin protein Z-line beta subunit (*CAPZB*)	Murrah	Xiong *et al*. (2018), China
	Heat shock protein70 (*HSP70*)	Egyptian	Gafer *et al*. (2015), Egypt
	Prolactin (*PRL*)	Egyptian	Hasanain *et al*. (2017), Egypt
3	Growth hormone (*GH*)	Egyptian	Darwish *et al*. (2016), Egypt
	Prohibitin (*PHB*)	Murrah	Xiong *et al*. (2018), China
	Aquaporin 7 (*AQP7*)	Murrah	Kumari *et al*. (2018), India
	Gonadotropin-releasing hormone (*GnRH*)	Chinese water	Wang *et al*. (2020), China
6	Tektin-2 (*TEKT2*)	Murrah	Xiong *et al*. (2018), China
7	Secreted phosphoprotein 1 (*SPP1*)	Brazilian Water	Rolim Filho *et al*. (2013), Brazil
	Gonadotropin releasing hormone receptor (*GnRHR*)	Chinese water	Wang *et al*. (2017), China
		Egyptian	Mahmoud *et al*. (2021), Egypt
8	Leptin (*LEP*)	Nili-Ravi	Dilbar *et al*. (2019), Pakistan
11	AT-rich interaction domain 4A (*ARID4A*)	Chinese water	Lu *et al*. (2022), China
12	Luteinizing hormone receptor (*LHR*)	Murrah	Reen *et al*. (2018), India
16	Ubiquilin-3	Egyptian	EL Nagar *et al*. (2023), Egypt
17	Inhibit alpha (*INHA*)	Murrah	Chandra *et al*. (2020), India.
18	Luteinizing hormone beta (*LHβ*)	Murrah	Reen *et al*. (2018), India
X or 25	Melanoma-associated antigen D2 gene, Cancer/testis antigen 47A-like gene, Actin-related protein T1 gene, Sodium/hydrogen exchanger 2-like gene	Egyptian	EL Nagar et al. (2023), Egypt

CN= Chromosome number

2.11 Candidate genes and their molecular associations with growth traits in buffalo

For growth traits, strong molecular associations of candidate genes with body weights and gains in buffalo were reported in several buffalo studies (**Table 18).** According to the available molecular results mentioned in **table 18** along with those genes listed in GWAS literature **(Guzman *et al*., 2020; Rehman *et al*., 2022; Khan *et al*., 2022**), body weights and gains in buffalo are significantly controlled by the following candidate genes. On chromosome 1, several genes are associated with growth and carcass traits like Pituitary-specific transcription factor-1 gene (*Pit1*) is associated with body weight in Khuzestan water buffalo **(Ahmadzadeh *et al*. 2019)** and Egyptian buffalo **(Othman *et al*, 2012)**. On chromosome 2, Prolactin gene (*PRL*) was detected in Egyptian buffalo **(Abd El Fattah *et al*., 2023)**, Myostatin gene (*MSTN*) was detected in Murrah buffalo **(Páez *et al*., 2021)** and Signal transducer and activator of transcription 1 gene (*STAT1*) was detected in Chinese water buffalo (**Deng *et al*., 2016**). On chromosome 3, strong associations were detected between growth traits and Growth hormone gene (*GH*) in Indonesian buffalo **(Andreas *et al*., 2010)**, Anatolian water buffalo (**Konca and Akyüz, 2017; Özkan Ünal *et al*, 2020),** Khuzestan water buffalo **(Ahmadzadeh *et al*, 2019),** Simeulue buffalo (**Eriani *et al*., 2019)** and Swamp buffalo **(Nafiu *et al*., 2020)**. On chromosome 5, significant associations were detected between growth traits and Insulin-like growth factor 1 and 2 genes (*IGF1 or IGF2*) in Egyptian buffalo **(Abo Al-Ela *et al*., 2014; El-Magd *et al*., 2014)** and Asian water buffalo **(Ramesha *et al*., 2015)**. Strong associations were also detected between growth traits and Kappa-casein N3 gene (*KCN3*) located on chromosome 7 in Khuzestan water buffalo (**Ahmadzadeh *et al*., 2019)**, Leptin gene (*LEP*) located on chromosome 8 in Anatolian water buffalo **(Kaplan, 2018)** and Insulin-like growth factor binding protein-3 gene (*IGF*BP-*3*) in Egyptian buffalo **(Othman *et al*., 2018)** and Asian water buffalo **(Ramesha *et al*., 2015)**. The other molecular associations of growth and/or carcass traits were detected on chromosome 10 for Insulin-like growth factor 1 receptor gene (*IGF1)* and Insulin-like growth

factor 2 receptor gene (*IGF2R*) in Egyptian buffalo (**El-Magd *et al*., 2014, 2017).**

Table 18. The list of candidate genes molecularly associated with body weights and gains as cited in buffalo literature

CN	Candidate gene	Buffalo breed	Reference and country of work
1	Pituitary-specific transcription factor-1 *(Pit1*)	Egyptian	Othman *et al*. (2012), Egypt
		Khuzestan	Ahmadzadeh *et al*. (2019), Iran
2	Prolactin (*PRL*)	Egyptian	Abd El Fattah *et al*. (2023), Egypt
	Myostatin (*MSTN*)	Murrah	Páez *et al*. (2021), Colombia
	Signal transducer and activator of transcription 1(*STAT1*)	Chinese	Deng *et al*. (2016), China
3	Growth hormone (*GH*)	Indonesian	Andreas *et al*. (2010), Indonesia
		Anatolian	Konca and Akyüz. (2017), Turkey
		Khuzestan	Ahmadzadeh *et al*. (2019), Iran
		Simeulue	Eriani *et al*. (2019), Indonesia
		Anatolian	Özkan Ünal *et al*. (2020), Turkey
		Swamp	Nafiu *et al*. (2020), Indonesia
5	Insulin-like growth factor 1 (*IGF-1*)	Asian	Ramesha *et al*. (2015), India
		Egyptian	El-Magd *et al*. (2017), Egypt
	Insulin-like growth factor 2 (*IGF2*)	Egyptian	El-Magd *et al*. (2014), Egypt
		Egyptian	Abo Al-Ela *et al*. (2014), Egypt
7	Kappa-Casein N3 (*KCN3*)	Khuzestan	Ahmadzadeh *et al*. (2019), Iran
8	Leptin (*LEP*)	Egyptian	Othman *et al*. (2011a), Egypt
		Anatolian	Kaplan (2018), Turkey
	Insulin-like growth factor binding protein-3 (*IGF*BP-*3*)	Asian	Ramesha *et al*. (2015), India
		Egyptian	Othman *et al*. (2018), Egypt
10	Insulin-like growth factor 2 receptor (*IGF2R*)	Egyptian	El-Magd *et al*. (2014), Egypt
	Insulin-like growth factor 1 receptor (*IGF1R*)	Egyptian	El-Magd *et al*. (2017), Egypt
14	Growth hormone releasing hormone (*GHRH*)	Asian	Ramesha *et al*. (2015), India
		Anatolian	Konca and Akyüz. (2017), Turkey
		Khuzestan	Ahmadzadeh *et al*. (2019), Iran
		Swamp	Nafiu *et al*. (2020), Indonesia
17	Calpain 1 (*CAPN1*)	Egyptian	Othman *et al*. (2010), Egypt
	Inhibin-βA (*INHβA*)	Philippine	Babera *et al*. (2022), Philippine
19	Growth hormone receptor (*GHR*)	Indonesian	Andreas *et al*. (2010), Indonesia
		Anatolian	Erdoğan *et al*. (2021), Turkey
	Prolactin-like receptor (*PRLR*)	Egyptian	Abd El Fattah *et al*. (2023), Egypt

CN= Chromosome number

on chromosome 14 for Growth hormone releasing hormone gene (*GHRH*) in Asian water buffalo (**Ramesha *et al*., 2015),** Anatolian water buffalo **(Konca and Akyüz., 2017),** Khuzestan water buffalo **(Ahmadzadeh *et al*. 2019)** and Swamp buffalo **(Nafiu *et al*., 2020),** and on chromosome 17 for Calpain 1 gene (*CAPN1*) in Egyptian buffalo (**Othman *et al*., 2010)** and Inhibin-βA gene (*INHβA*) in Philippines water buffalo **(Babera *et al*., 2022**). On chromosome 19, Growth hormone receptor gene (*GHR*) is associated with body weights, daily gains and consequently on growth, carcass and meat composition in Indonesian buffalo (**Andreas *et al*., 2010)** and Anatolian buffalo **(Erdoğan *et al*. 2021).** In addition, Prolactin-like receptor gene (*PRLR*) was investigated in Egyptian buffalo **(Abd El Fattah *et al*., 2023)**.

2.12 Molecular characterization of prolactin gene (*PRL*, as a functional candidate gene) in buffalo

The *PRL* gene was mapped on chromosome 2 in buffalo and composed of 10 exons (of which exon 1 and 2 are non-coding) **(Hu *et al*., 2009; Lü *et al*., 2011)**.

2.12.1 Molecular weights for *PRL* gene

The amplified fragments obtained by **Ladani *et al*. (2003)** in Meshing buffalo evidenced the presence of three different genotypes of *PRL* gene; two homozygous genotypes of one undigested fragment at 156 *bp* and two digested fragments with sites of 74 and 82 *bp* and one heterozygous genotype of three digested fragments with sites of 74, 82 and 156 bp. **Mavi *et al*. (2017)** in Murrah buffalo found one homozygous genotype for *PRL* gene (with fragment length of 294 bp). In Anatolian water buffalo, **Konca and Akyüz (2017)** reported one undigested fragment 156 *bp* for *PRL* gene, while the two digested fragments with length of 82 and 74 *bp* and three fragments with length of 156, 82 and 74 *bp* for heterozygous genotype. **Hasanain *et al*. (2017)** in Egyptian buffalo found one undigested fragment of 678 *bp* of *PRL* gene and two digested fragments with length of 231 *bp* and 447 bp. **Özşensoy**

(2018) reported two homozygous genotype of one undigested 156 *bp* fragment of *PRL* gene and two digested fragments of 82 and 74 bp, while the fragments with length of 156, 82 and 74 *bp* indicated for heterozygous genotype.

2.12.2 The effective number of alleles (N_e), *Hardy-Weinberg equilibrium (HWE) and* polymorphic information content (PIC) for *PRL* gene

El-Magd *et al*. (2015) in Egyptian buffalo stated that the effective number of alleles (*Ne*) for *PRL* gene was 1.759 and the value of polymorphic information content (*PIC*) was moderate (0.338). Chi-square value was high (41.9), indicating that the population of Egyptian buffalo was not in *HWE* and this high deviation in *HWE* suggests change in the distribution of alleles from one generation to the next generations. Depending on the number of detectable alleles and the distribution of their frequency, the value of *PIC* gives an estimate of the markers discriminating power and thus, describes the markers usefulness for identifying polymorphism within a buffalo population (**El-Magd *et al*., 2015)**. **Konca and Akyüz (2017)** showed that the value of Chi-square for genotypes of *PRL* gene in the Anatolian water buffalo was high (50.63), indicating that this population was not in HWE.

2.12.3 Genotypic and allelic frequencies for genotypes of *PRL* gene

Ladani *et al*. (2003) stated that frequency of A allele for *PRL* gene in Jaffarabadi, Mehsani and Surti buffaloes were 0.435, 0.5 and 0.482, while the frequency of AA genotypes were 0.565, 0.5 and 0.518, respectively. **Ishaq *et al*. (2013)** using PCR-RFLP technique, examined the *PRL* gene polymorphisms in Nili-Ravi, Sahiwal, and Akai buffalo and reported that one genotype of GG was detected in Nili-Ravi buffalo, three genotypes of AA, AG and GG were detected in Sahiwal and Achai buffalo with frequencies of 0.72, 0.18 and 0.10 in Sahiwal buffalo and 0.44, 0.34 and 0.22 in Achai buffalo, respectively. **El-Magd *et al*. (2015)** reported that the allele frequency for C allele was 0.315 and it was 0.685 for T allele in Egyptian buffalo and accordingly the genotypic frequencies were 0.37 for CC genotypes and 0.63 for CT genotype. In the Anatolian water buffalo, **Konca and Akyüz (2017)**

reported that the allele frequency of *PRL* gene was 0.55 for A allele and 0.45 for B allele, while the genotypic frequencies for AA, AB and BB genotypes were 0.143, 0.81 and 0.047, respectively. But in other study, **Özşensoy (2018)** in the Anatolian water buffalo reported that the allele frequency of *PRL* gene was 1.0 for A allele and 0.0 for B allele (monomorphic).

2.12.4 Heterozygosis of *PRL* gene

El-Magd *et al*. (2015) found that the level of heterozygosity was high (0.431) for *PRL* gene in Egyptian buffalo. **Nadeem and Maryam (2016)** stated that the observed (*Ho*) and expected (*He)* heterozygosities for *PRL* gene in Nili-Ravi buffalo were 0.8159 and 0.1841, respectively.

2.13 Molecular characterization of diacylglycerol O-acyltransferase 1 gene (*DGAT1*, as a functional candidate gene) in buffalo

The *DGAT1* gene is mapped on chromosome number 15 in buffalo and composed on 17 exons.

2.13.1 Molecular weights for *DGAT1* gene

Yuan *et al*. (2007) in Chinese buffalo reported that the range in size of *DGAT1* gene was from 160 *bp* to 300 *bp*. But**, Özdil and Ilhan (2012)** in Anatolian buffalo reported that the undigested fragment of *DGAT1* gene at 411 *bp* was indicated for GG genotype, while the digested fragments at 176, 167 and 68 bp*s* were indicated for CC genotype and the fragments at 411, 167, 137 and 107 bp*s* were indicated for heterozygous GC genotype. **Freitas *et al*. (2016)** showed that the PCR fragment was 231 *bp* for *DGAT1* gene in the Murrah buffalo.

2.13.2 The effective number of alleles (N_e) and *Hardy-Weinberg equilibrium (HWE)* for *DGAT1* gene

Sulabh *et al*. (2018) in Murrah buffalo stated that the effective numbers of alleles (Ne) for five *SNPs* of *DGAT1* gene were 1.298, 1.237, 1.433, 1.367 and 1.197 with moderate *PIC* values to be 0.226, 0.192, 0.264, 0.234 and 0.217.

Freitas *et al*. (2016) in Murrah buffalo found that Chi-square values for genotypes of *DGAT1* gene were low for the two *SNPs* identified (1.24 and 1.94), indicating that this population of buffalo was in *HWE* for this gene. **Silva *et al*. (2016)** in Murrah and Mediterranean buffalo revealed that Chi-square values for *HWE* of *DGAT1* gene were low (0.70 and 0.67) and therefore both populations were in HWE.

2.13.3 Genotypic and allelic frequencies for genotypes of *DGAT1* gene

Yuan *et al*. (2007) reported that the allele frequencies of A and B alleles of *DGAT1* gene in Chinese buffalo were 0.823 and 0.177 with genotypic frequencies of 0.646, 0.354 and 0.00 for AA, AB and BB genotypes, respectively. **Heydarian *et al*. (2014)** found that the allelic frequencies for A allele of *DGAT1* gene in Mazandaran, Khuzestan, Guilan, Azerbayjan and Kermanshah buffalo were 0.78, 0.68, 0.80, 0.60 and 0.73, while they were 0.22, 0.32, 0.20, 0.40 and 0.27 for B allele and therefore the genotypic frequencies were 0.56, 0.38, 0.65, 0.20 and 0.56 for AA genotype, 0.44, 0.62, 0.35, 0.78 and 0.0 for AB genotype, and 0.0, 0.0, 0.44, 0.0 and 0.02 for BB genotype, respectively. **Freitas *et al*. (2016)** in Murrah buffalo reported that the allele frequency was 0.07 for A allele of *DGAT1* gene and 0.93 for G allele, while the genotypic frequencies were 0.0, 0.14 and 0.86 for AA, AG and GG genotypes and the allele frequency was 0.40 for C allele and 0.60 for T allele, while the genotypic frequencies were 0.34, 0.53 and 0.13 for CC, CT and TT genotypes, respectively. **Silva *et al*. (2016)** found that the allele frequency for A allele of *DGAT1* gene was 0.69 and it was 0.31 for B allele in Mediterranean buffalo, while the genotypic frequencies for AA, AB and BB genotypes were 0.44, 0.50 and 0.06, respectively. **Kadhim and Ibrahim (2019)** found that the allele frequencies of C and T alleles of *DGAT1* gene in Iraqi buffalo were 0.12 and 0.88 with genotypic frequencies of 0.23 and 0.77 for CT and TT genotypes, respectively**.**

2.13.4 Heterozygosis of *DGAT1* gene

Silva *et al*. (2016) stated that the observed heterozygosity (*Ho*) in Murrah and Mediterranean buffaloes were 0.68 and 0.50, while the expected heterozygosity (*He*) were 0.34 and 0.43, respectively with the reduction in heterozygosity due to inbreeding (F_{IS}) to be 0.78 and -0.15 in Murrah and Mediterranean buffalo, *i.e.* these two buffalo populations showed high levels of genetic diversity in *DGAT1* gene for milk production.

2.14 Molecular characterization of follicle-stimulating hormone receptor gene (*FSHR*, as a functional candidate gene) in buffalo

The *FSHR* gene is mapped on chromosome 12 in buffalo and composed of 10 exons and nine introns (**Simoni *et al.*, 1997**).

2.14.1 Molecular weights of *FSHR* gene

Othman and Abdel-samad (2013) in PCR amplified fragments (306 bp) differentiate between three genotypes of *FSHR* gene (CC, CG and GG) (306 bp) in Egyptian buffalo and identified that two digested fragments using *AluI endonuclease* restriction enzyme at 243 and 63 *bp* for CC genotype, three digested fragments at 193, 63 and 50 *bp* for GG genotype and four digested fragments at 243, 193, 63 and 50 *bp* for CG genotype. By using *AluI endonuclease* for digestion of 306 *bp* product, **Sosa *et al*. (2015)** differentiated between three different genotypes of *FSHR* gene in Egyptian buffalo (CC, TT and CT) and reported that two digested fragments at 243 and 63 *bp* for CC genotype, three digested fragments at 193, 63 and 50 bps for GG genotype and four digested fragments at 243, 193, 63 and 50 *bp* for CG genotype. **Shafik *et al*. (2017)** showed that there was one non synonymous *SNP* (A93G) in Egyptian buffalo at 93 *bp* in exon 10 of *FSHR* gene (with 230 *bp* size)**.** In Murrah buffalo**, Kathiravan *et al*. (2019)** found that the PCR product of *FSHR* gene (exon 10) was monomorphic, with bands of 243 and 63 *bp* corresponding to CC genotype and representing the presence of C allele.

2.14.2 *Hardy-Weinberg equilibrium (HWE) and* polymorphism information content (PIC) for *FSHR* gene

Fouda *et al*. (2021) stated that Chi-square values for genotypes of *FSHR* gene (GG and CG) in Egyptian buffalo were moderate (3.948 and 7.852), indicating that this population was not in HWE. **Setyorini *et al*., (2023)** reported that the value of Chi-square for genotypes of *FSHR* gene was high (3.2), indicating that *FSHR* gene in Indonesian Holstein dairy cattle was not in HWE.

Putra *et al*. (2020) stated that the PIC values for bovine *FSHR* gene in Indonesian Pasundan cattle were moderate and ranged from 0.30 to 0.50. Moreover, in Zebu × British composite crossbred cattle and indigenous Turkish breed, the PIC values were also moderate, being 0.37 and 0.34, respectively (**Marson *et al*., 2008**; **Arslan *et al*., 2015).**

2.14.3 Genotypic and allelic frequencies for genotypes of *FSHR* gene

In Egyptian buffalo, **Othman and Abdel-samad (2013)** reported that 100% of the animals investigated had the same genotype of *FSHR* gene (monomorphic). Also, **Shafik *et al*. (2017)** stated that the frequencies for A and G alleles of *FSHR* gene were 0.014 and 0.985 along with genotypic frequencies of 0.00, 0.028 and 0.972 for AA, AG and GG genotypes, respectively. Moreover, **Fouda *et al*. (2021)** reported that the allele frequencies of the C and G alleles in Egyptian buffalo were 0.54 and 0.46 with genotypic frequencies of 0.34, 0.40 and 0.26 for CC, CG and GG genotypes, respectively. **Kathiravan *et al*. (2019)** in Murrah buffalo found that the allelic frequency of genotype CC of *FSHR* gene was monomorphic (100%) and the allelic frequency was 1.0 for allele C and 0.0 for T allele.

2.14.4 Heterozygosis of *FSHR* gene

Setyorini *et al*., (2023) in Indonesian Holstein dairy cattle found that the value of observed heterozygosity was 0.490 and the value of expected heterozygosity was 0.416 for *FSHR* gene.

2.15 Molecular characterization of growth hormone gene (*GH*, as a functional candidate gene) in buffalo:

The *GH* gene is located on autosomal chromosome number 3 and the *GH* gene structure in buffalo is unknown (**Andreas *et al*., 2010; Konca and Akyüz, 2017; Ahmadzadeh *et al*., 2019; Özkan Ünal *et al*., 2020; Nafiu *et al*., 2020)**.

2.15.1 Molecular weights of *GH* gene

Othman *et al*. (2012) in Egyptian buffalo found that one fragment of 211 *bp* was identified for the homozygous VV genotype of *GH* gene and two fragments were digested by *AluI endonuclease* restriction enzyme at 159 *bp* and 52 *bp* for LL and LV genotypes. **Konca and Akyüz (2017)** in Anatolian water buffalo reported that the undigested fragment at 211 *bp* of *GH* gene indicated for VV genotype, while the digested fragments at 159 and 52 *bp* indicated for LL genotype and the fragments at 211, 159 and 52 *bp* were indicated for heterozygous LV genotype. **Nafiu *et al*. (2020)** found that the undigested 327 bps fragment of *GH* gene in Swamp buffalo was indicated for BB genotype, while the digested fragments at 104 and 223 *bp* indicated for AA genotype and the fragments at 104, 223 and 327 *bp* indicated for the heterozygous AB genotype.

2.15.2 *Hardy-Weinberg equilibrium (HWE)* for *GH* gene

Konca and Akyüz (2017) reported that the value of Chi-square for genotypes of *GH* gene was low (0.02), indicating that *GH* gene in Anatolian water buffalo was in *HWE*. **Nafiu *et al*. (2020)** in the Swamp buffaloes, stated that Chi-square value for *GH* gene genotypes was also low (0.89), indicating that this population was in *HWE*.

2.15.3 Genotypic and allelic frequencies for genotypes of *GH* gene

Andreas *et al*. (2010) reported that the allelic frequency of *GH* gene for L allele was 1.0 and 0.0 for V allele with genotypic frequency was 1.0 (monomorphic) for LL genotype and 0.0 for VV and VL genotypes in Indonesian buffalo. **Konca and Akyüz (2017)** reported that the allele

frequency in Anatolian buffalo was 0.87 for L allele and 0.13 for V allele, while the genotypic frequencies were 0.755, 0.228 and 0.017 for LL, LV and VV genotypes, respectively. **Eriani *et al.* (2019)** stated that the frequency of *GH* gene in Simeulue buffalo was 0.533 for A allele and 0.467 for B allele, with genotypic frequencies of 0.133, 0.866 and 0.066 for AA, AB and BB genotypes, respectively. **Nafiu *et al.* (2020)** found that frequency of A allele for *GH* gene in Indonesian Swamp buffalo was 0.562, while the frequency for B allele was 0.438, with genotypic frequencies of 0.375, 0.375 and 0.250 for AA, AB and BB genotypes, respectively. **Anggraeni *et al.* (2023)** stated that the genotypic frequency of *GH* gene in Indonesian Swamp buffalo was monomorphic (100%) for TT genotype and 0% for TC and CC genotypes, with allelic frequency of 1.0 for T allele and 0.0 for C allele.

2.15.4 Heterozygosis in genotypes of *GH* gene

Eriani *et al.* (2019) in Indonesian buffalo found that the value of observed heterozygosity *(H_O)* was 0.80 and the value of expected heterozygosity *(He)* was 0.49. **Nafiu *et al.* (2020)** in Swamp buffalo found that the *Ho* value was 0.375 and the value of *He* was 0.492. **Anggraeni *et al.* (2023)** in Indonesian Swamp buffalo reported that *Ho* and *He* values for *GH* gene were equal to 0.0 (monomorphic).

2.16 Molecular association of prolactin gene *(PRL,* as a functional candidate gene) with lactation, reproduction, growth and semen traits in buffalo

The molecular studies have shown that *PRL* gene can be used as a candidate gene for the genetic improvement of milk production and composition characteristics in buffalo **(Nadeem and Maryam, 2016** in Pakistan**; Li *et al.*, 2017** in China**; Konca and Akyüz., 2017 and Özşensoy, 2018** in Turkey**; Mavi *et al.*, 2017** in India**; El-Komy *et al.*, 2020** in Egypt**)**. These molecular studies are encouraging factors to use *PRL* gene as a candidate gene for identifying the molecular markers associated with lactation traits in buffalo. However, *PRL* gene is known to have various biological functions such as water and electrolyte balance, growth and development,

immune and reproduction function **(Gregerson, 2006)**. Also, *PRL* gene plays a central role in mammalian reproduction, glandular development, milk secretion, and expression of milk protein. In Murrah buffalo**, Singh *et al*. (2015)** found that *PRL* gene is an important candidate gene known to be associated with milk production traits as well as somatic cell counts (SCC).

To our knowledge, there are no previous studies concerning the molecular association between *PRL* gene and growth traits in buffalo although there are limited studies in cattle. In Angus cattle, **Meyer *et al*. (2017)** demonstrated that genotypes of the *PRL* gene impacted significantly heavier live body weights of calves at birth and weaning.

In Egyptian buffalo, **Hasanain *et al*. (2017)** showed that *PRL* gene is an important candidate gene known to be associated with ejaculate volume, individual motility and live sperm percentage significantly in favor of AA and BB genotypes.

2.17 Molecular association of diacylglycerol O-acyltransferase1 gene (*DGAT1*, as a functional candidate gene) with lactation, reproduction, growth and semen traits in buffalo

Liu *et al*. (2020) performed a comprehensive analysis for the DGAT family genes in buffalo, which including identification, structural characterization, phylogenetic classification, chromosomal distribution, association analysis, and functional analysis and determine the role of *DGAT* family genes in regulation of milk production and milk quality improvement in buffalo. *DGAT1* gene was known to control the rate of triglyceride synthesis via adipocytes and in considering the influence of the fatty acids contents in milk **(Yuan *et al*., 2007; Tăbăran *et al*., 2015; Liu *et al*., 2020)**. It was verified to be associated with lactation and/or reproduction traits in Chinese buffalo (**Yuan *et al*., 2007),** in Anatolian buffalo **(Özdil and Ilhan, 2012),** in Murrah buffalo (**Freitas *et al*., 2016),** in Riverine buffalo **(Li *et al*., 2017)** and in Egyptian buffalo **(El-Komy *et al*., 2020)**. In Mehsana buffalo, **Parikh *et al*. (2016)** reported that SNP of AA and GA genotypes of *DGAT1* gene had significantly (*P<0.01*) higher milk yield (2169 and 2363 kg) than GG genotype (1577 kg). In Murrah buffalo, **Sulabh *et al*. (2018)** found that

the *SNPs* of *DGAT1* gene was significantly (P < 0.05) associated with fat yield where AG genotype was the most favoured, i.e the *SNPs* g.A7013G of *DGAT1* gene may be used as a potential marker for selection favouring to increase fat yield. **Li *et al*. (2018c)** revealed that the *SNP* (g.9046T>C) of *DGAT1* gene was significantly associated with fat percentage i.e TT genotype had significant higher means for fat percentage than CC genotype in Riverine and Swamp buffaloes. **Kadhim and Ibrahim (2019)** revealed that *DGAT1* gene affected significantly daily milk production in Iraqi buffalo, where CT genotype had the highest milk production compared with TT genotype and non-significant for protein and fat percent.

2.18 Molecular association of follicle-stimulating hormone receptor gene (*FSHR*, as a functional candidate gene) with lactation, reproduction and semen traits in buffalo

Several Egyptian studies have shown that the *FSHR* gene is considered as an important candidate gene for reproduction and fertility traits in Egyptian buffalo (**Othman and Abdel-samad, 2013; Shafik *et al*., 2017; Ramadan *et al*., 2020; Fouda *et al*., 2021; Sallam *et al*., 2022)**. In these Egyptian studies, **Shafik *et al*. (2017)** found significant association between *FSHR* gene and calving interval, days open, dry period, days in milk, total milk yield and 305-day milk yield. Also, **Sallam *et al*. (2022)** reported significant association between *FSHR* gene and sperm motility.

2.19 Molecular association of growth hormone gene (G*H*, as a functional candidate gene) with lactation, reproduction, growth and semen traits in buffalo

The molecular Egyptian studies have shown that *GH* gene can be used as a candidate gene for genetic improvement of growth traits in buffalo since this gene is known to have various biological functions such as water and electrolyte balance, milk production and reproduction functions **(Othman *et al*., 2012; Darwish *et al*., 2016)**. Other Non-Egyptian studies have shown that there are polymorphic associations between *GH* gene as a candidate gene and growth, carcass, and semen quality traits in buffalo (***e.g***. **Konca and Akyüz, 2017; Özkan Ünal *et al*. 2020; Nafiu *et al*., 2020)**. Also, **Eriani *et al*. (2019)** found a significant association between *GH* gene and body size in Simeulue buffalo.

3. MATERIALS AND METHODS

3.1 Buffalo herds studied:

Six experimental buffalo herds, nominated as El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S), belong to the Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation (MALR), Egypt were used in this study. The herds NG and NK are located in Kafr El-Sheikh Governorate, while EN herd is located in Behira Governorate, EG herd in Gharbia Governorate, ES herd in Damietta Governorate and S herd in Beni Suef Governorate. All the herds are located in the Nile Delta region, lower Egypt except Sids herd is located in Upper Egypt.

The bulls aged 18 to 24 months with scrotal size of more than 19 cm were used for insemination. These bulls were raised in two herds of the International Livestock Management Training Center at Sakha (IMTC) and Mahalet Mousa (MM), Kafr El-Sheikh Governorate, belonging to Animal Production Research Institute (APRI), Agriculture Research Center, Ministry of Agriculture, Egypt. All the bulls were free of any clinical diseases with healthy appearances. The semen was collected individually from each bull at 8 AM using an artificial vagina (IMV, France).

3.2 Management and feeding

Buffaloes were kept under semi-open sheds; heifers were joined for the first service when reaching 24 months of age or 330 kg body weight. Buffaloes were naturally mated in a group-mating system and in few cases the buffaloes were artificially inseminated. Rectal palpation was applied to check pregnancy at 60 days post-mating. Milking was practiced twice a day at 7 AM and 4 PM throughout the lactation period. Buffaloes were fed Egyptian Berseem (*Trifolium alexandrinum*) along with varying amounts of integrated concentrate feed mixture (48% decorticated cotton seed cake, 21% wheat bran, 20 % maize, 5 % rice polish, 3 % molasses, 2 % limestone, and 1

% sodium chloride) according to APRI feeding routine. The diet contains 16 % protein for breeding buffaloes and heifers and 17 % protein for suckling calves during the period from 105 days of age. Feed is offered manually starting with the roughage (silage - rice straw - alfalfa – alfalfa hay), followed by the concentrate feed. Feeding takes place twice a day at six AM and then at five PM and clean water is available all the time. The amount of feed required for each animal was calculated depending on the animal weight and quantity of daily milk produced. The calves were weighed immediately after birth and then weighed monthly. Buffaloes were dried off two months before the expected day of calving. The abnormal lactations or reproduction records affected by diseases or having missing birth dates, dry off dates or yields were excluded.

Buffalo bulls were daily fed on a ration consisting of 4 kg concentrate feed mixture (48 % decorticated cotton seed cake, 21 % wheat bran, 20 % maize, 5 % rice polish, 3 % molasses, 2 % limestone, and 1 % sodium chloride), 3 kg clover hay, and 4 kg rice straw. The ration was offered twice daily and clean water was available all the time.

The calves were fed colostrum for the first three days after birth at 3% of their body weight, weighed individually within the same day of birth to record birth weight (BW, *kg*), and weighed to record weaning weight (WW, *kg*) after 105 days from birth. To obtain daily weight gain (DG, *kg*/d), BW was subtracted from WW and then divided by 105.

Buffaloes were regularly vaccinated against foot and mouth disease at four months interval and yearly against Clostridia, Pasteurelloses and three-day fever.

3.3 Data structure of lactation traits

A total number of 7345 test-day records (TD) of milk, fat and protein yields and somatic cell scores were gathered monthly from 686 buffaloes, daughters of 83 sires and 423 dams for a period of 21 years starting from 2003 up to 2023 in three experimental buffalo herds of NG, NK and EG. Records of TD milk were collected following an alternative AM: PM

monthly recording scheme. The buffaloes having abnormal phenotypic values for daily milk yield or less than four TD records per lactation were excluded from the milk data set. The maximum number of TD milk records per lactation per buffalo cow was nine. All available relationships among animals were considered in the statistical analyses. The pedigree file comprised a total of 10802 relationship records was used. The number of buffalo animals and records belonging to the three studied herds used in data analyses for lactation traits are shown in **Table 19**.

Table 19. Summary of the data available for lactation traits used in data analysis of the three studied Egyptian buffalo herds

Item	NG herd	NK herd	EG herd	All herds
Number of buffaloes with records	212	440	34	686
Number of sires with records	22	59	2	83
Number of dams with records	140	268	15	423
Total number of animals (buffaloes, sires and dams)	374	767	51	1192
Total number of test-day milk records	2201	4713	431	7345

NG= El-Nattafe El-Gadid herd, NK= El-Nattafe El-Kadim herd and EG= El-Gimmeza herd.

Data of TD lactation yields of milk (TDMY), fat (TDFY) and protein (TDPY) and somatic cell score (TDSCS) were used in the present study. TD records between five and 270 days in milk (DIM) were considered in the statistical analysis. The first TD included test days between four and 15 days in milk (DIM) and all the subsequent tests were classified as 30-d interval up to 270 DIM and therefore the buffaloes used in the analyses had at least four TD records per lactation. TD data after 270 days was discarded from the data file because it had few numbers of observations. TD records per lactation were classified into nine test-days (TD1 to TD9) according to days in milk. Fat and protein percentages as well as the somatic cell count were measured by the automated method of infrared absorption spectrophotometry (Milk-o-Scan; Foss Electric, Hillerφd, Denmark) at the Dairy Services Unit, Animal Production Research Institute, Sakha, Kafr El-Sheikh Governorate, Egypt. The somatic cell count (SCC) is recorded monthly in thousands per *ml* and

transformed to somatic cell score (SCS) using log^{10} scale to achieve an approximate normal distribution (**EL-Bramony *et al*., 2004**).

3.4 Data structure of reproduction traits

Records of age at first calving (AFC), days open (DO) and calving interval (CI) were collected from the database file of the six studied APRI herds. A total number of 7279 reproduction records collected for a period of 22 years (2002 to 2023) from 1951 buffaloes, daughters of 155 sires and 1179 dams were used in this study. Also, all available relationships among animals were considered in analyses of reproduction traits. The number of buffalo animals and records belonging to the six herds were used in data analyses of reproduction traits (**Table 20**).

Table 20. Summary of the data available for reproduction traits used in data analysis of the six studied Egyptian buffalo herds

Item	Herd						
	NG	NK	EN	S	EG	ES	All herds
Number of buffaloes with records	805	285	42	253	526	40	1951
Number of sires with records	75	30	8	13	25	4	155
Number of dams with records	479	187	27	159	309	18	1179
Total number of animals (buffaloes, sires and dams)	1359	502	77	425	860	62	3285
Total number of reproduction Records	3104	1278	103	861	1885	48	7279

NG= El-Nattafe El-Gadid herd, NK= El-Nattafe El-Kadim herd, EN= El-Nubaria herd, S= Sids herd, EG= El-Gimmeza herd and ES= El-Serw herd.

The differences among the numbers of animals and records for lactation traits relative to those for reproduction traits are attributed to the fact that the data related to the reproduction traits are easy to record each parturition to track any fertility disorders, while the data related to milk composition traits are lesser due to the cost of measuring milk composition.

3.5 Semen collection and evaluation

A total of 5178 semen ejaculates were collected from 111 Egyptian buffalo bulls (weighing 350–400 kg in live body weight) produced from 34

sires and 92 dams during 10 years from 2013 to 2022. Semen was collected using an artificial vagina set up at optimal conditions to induce good ejaculatory thrust. At the time of semen collection, another buffalo bull was used as a teaser for sexual preparation. Semen ejaculates were obtained from each bull once a week at early morning (8:00 AM) throughout four consecutive weeks during four seasons of the year. The ejaculates were taken immediately to the laboratory in a water bath at 37°C for semen evaluation processes. The number of buffalo animals and records belonging to the two studied herds used in data analyses for semen traits are shown in **Table 21.**

Table 21. The number of buffalo animals and records belonging to the two herds used in data analyses of semen traits

Item	**IMTC herd**	**MM herd**	**Both herds**
Number of bulls with records	7	104	111
Number of sires with progeny and records	5	29	34
Number of dams with progeny and records	5	87	92
Total number of animals (bulls, sires and dams)	17	220	237
Total number of semen records	566	4612	5178

IMTC= International Livestock Management Training Center herd at Sakha and MM= Mahalet Mousa herd

Using a graduated glass tube, the semen ejaculate volume was measured directly in milliliters to the nearest 0.1 *ml*. The sperms concentration in each semen ejaculate (10^9 sperms/*ml*) was determined using a Neubauer hemocytometer. The percentages of motile sperms were assessed using light microscope supplied with a hot stage adjusted to 37°C. Aliquots of evaluated semen were placed on the slide and covered by a warmed cover slip and were immediately examined under a high-power magnification (x400) according to **Vale *et al.* (2014)**. A smear from semen was made on a glass slide and stained by 1.67 % eosin (E 8761) and 10 % nigrosin (N 4763), attaining a mixture stain proposed by **Vale *et al.* (2014)**. All the experimental reagents used were purchased from Sigma-Aldrich (S.A., Egypt). About 10 g nigrosin and 1.67 g Eosin were dissolved in distilled water up to 100 *ml* for

the preparation of Eosin-Nigrosin stain at 37°C. One drop of the prepared stain was added to one drop of fresh semen and was mixed on glass slide at 37°C. Then, a thin smear was made by drawing of a second slide across the stained semen. The slide was allowed to be dried on the hot stage and then examined under a high-power magnification (x400). During the examination of live/dead sperms percentage at a high-power magnification (x400), the morphological screening of sperms was carried out per 200 sperms. The percentage of sperms wave motion in a drop of semen deposited on a glass slide was used to calculate semen mass motility (%). The abnormal sperms (%) were measured according to the procedure adopted by **Barbas and Mascarenhas (2009)**. Percentage of livability, motility of sperms and sperms count were estimated using a warm microscope stage in post-diluted, post-equilibrated and post-thawed semen adjusted at 37 d. The livability percentage of sperm cells was assessed by using eosin and nigrosine combination stain **(Vale *et al.*, 2014)**. Dead sperms (stained ones) and live sperms (unstained ones) were counted at field of 200 sperm cells.

3.6 Data structure of growth traits

Body weight at birth (BW) and weaning (WW) and daily weight gain (DG) were collected from the APRI database file of the six buffalo experimental herds (NG, NK, EN, ES, EG and S). Data on body weight were collected from 8229 buffalo calves, progeny of 277 sires and 2175 dams for a period of 22 years from 2003 to 2024. The numbers of calves and records in the pedigree and data files in different herds are shown in **Table 22**. All available relationships among animals were considered in the analyses.

Table 22. Number of Egyptian buffalo animals in the pedigree file used in genetic analyses for body weight and gain in six herds

Item	Herd						
	NK	NG	EN	EG	ES	S	All herds
Number of calves with records	1375	3591	95	2105	64	999	8229
Number of sires with records	49	123	24	48	8	25	277
Number of dams with records	329	917	39	562	41	287	2175
Total number of calves with records and sires and dams without records	1753	4631	158	2715	113	1311	10681

NG= El-Nattafe El-Gadid herd, NK= El-Nattafe El-Kadim herd, N= Nubaria herd, G= El-Gimmeza herd, ES= EL-Serw herd and S=Sids herd.

3.7 Models of quantitative genetics analyses

3.7.1 Animal Model and Random Regression Model used for analyzing lactation traits

The variance-covariance components of the random effects were estimated for TD milk, fat and protein yields and somatic cell score using TM software of Bayesian Gibbs Sampling Algorithm (**Legarra *et al.*, 2008**). The estimates obtained by Gibbs Sampling were used to solve the corresponding mixed model equations, using the PEST software to obtain the generalized least-squares means (GLSM) for TD lactation traits (**Groeneveld, 2006**). Therefore, the following single-trait repeatability animal model was used (Model 1):

$$\mathbf{y = Xb + Z_a u_a + Z_p u_p + e}$$ **(Model 1 Repeatability Single-trait animal model)**

Where: Y = the recorded lactation trait; b = vector of the fixed effects of herd-year test-day (271 levels), parity (5 levels), season of calving (4 levels) and covariable of days in milk (DIM); u_a = the vector of random additive genetic effects of buffaloes; up = the vector of random non-additive permanent environmental effects of buffaloes; X, Z_a and Z_p = incidence matrices for fixed effects, random additive genetic effects and random permanent environmental effects, respectively; e = vector of random error.

The variance-covariance components of the random effects were estimated using the following matrices:

$$Var\begin{bmatrix} a \\ p \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I_p\sigma_P^2 & 0 \\ 0 & 0 & I_n\sigma_e^2 \end{bmatrix}$$

Where: A = Numerator relationship matrix, I_p and I_n = identity matrix with order equal to number of animals and number of records, respectively, $\boldsymbol{\sigma^2}_{\boldsymbol{a}}$, $\boldsymbol{\sigma^2}_{\boldsymbol{P}}$ and $\boldsymbol{\sigma^2}_{\boldsymbol{e}}$ = the variances due to direct additive genetic effects, permanent environmental effects and random error, respectively. A single-trait repeatability animal model was used in analysis of lactation traits, considering the relationship coefficient matrix (A^{-1}) among the animals (**Korhonen, 1996**). The occurrence of local maxima was checked by repeatedly restarting the analyses until the log-likelihood did not change beyond the first decimal. Heritabilities (h^2) for TD lactation traits were computed using the TM software of Bayesian Gibbs Sampling Algorithm (**Legarra *et al.*, 2008**): $\boldsymbol{h^2} = \frac{\boldsymbol{\sigma^2}_{\boldsymbol{a}}}{\boldsymbol{\sigma^2}_{\boldsymbol{a}}+\boldsymbol{\sigma^2}_{\boldsymbol{P}}+\boldsymbol{\sigma^2}_{\boldsymbol{e}}}$, where $\boldsymbol{\sigma^2}_{\boldsymbol{a}}$, $\boldsymbol{\sigma^2}_{\boldsymbol{P}}$ and $\boldsymbol{\sigma^2}_{\boldsymbol{e}}$ as defined before.

For random regression model analysis (RRM), the VCE6 program was employed to analyze the data of TD lactation traits using the Legendre polynomials method. The variance-covariance components were estimated using the computer package VCE6 (**Groeneveld, 2010**) as (Model 2, RRM):

$$Y_{ijkl} = HTD_i + \sum_{m=1}^{4} \beta_{km} Z_{jlm} + \sum_{m=1}^{4} \alpha_{jm} Z_{jlm} + \sum_{m=1}^{4} \chi_{jm} Z_{jlm} + e_{ijkl} \quad (Model\ 2 - RRM)$$

Where: Yijk*l* = the test-day observation of yields of milk (TDMY), fat (TDFY) and protein (TDPY) or test-day somatic cell score (TDSCS) within l^{th} lactation made on i^{th} herd test-date (HTD_i) of the j^{th} buffalo cow belonging to k^{th} subclass TD (k ranged from 1 to 9 starting with k=1 equal 4 to 15 DIM and all the subsequent classes were classified as 30-d interval up to 270 DIM); HTD_i = the fixed effect of i^{th} herd test-day (114 levels), DIM = days in milk as linear and quadratic covariables; β_{km} = the fixed regression

coefficients for m^{th} TDMY or TDFY or TDPY or TDSCS on DIM of the k^{th} TD (year-season of calving, 80 levels and parity, 5 levels), α_{jm} = the random regression coefficients of additive genetic effects for m^{th} TDMY, TDFY, TDPY or TDSCC on DIM for j^{th} buffalo cow, x_{jm} = the random regression coefficients of permanent environment effects for m^{th} TDMY, TDFY, TDPY or TDSCC on DIM of the j^{th} buffalo cow; m= the number of traits (four traits); Z_{ilm} = the random genetic effect of TD lactation trait associated with all TD yields of the j^{th} buffalo cow and e_{ijkl} = random residual effect associated with Y_{ijkl}. Heritabilities for test day lactation traits were computed using RRM using VCE6 software as (**Groeneveld, 2010**): $\boldsymbol{h^2} = \frac{\boldsymbol{\sigma^2}_a}{\boldsymbol{\sigma^2}_a+\boldsymbol{\sigma^2}_P+\boldsymbol{\sigma^2}_e}$, where $\boldsymbol{\sigma^2}_a$, $\boldsymbol{\sigma^2}_P$ and $\boldsymbol{\sigma^2}_e$ as defined in Model 1.

3.7.2 Animal model for analyzing reproduction traits

The systematic environmental effects on DO and CI traits were evaluated using linear model fitting the fixed effects to avoid over-parameterization in the model. The variance components of random effects and heritabilities were estimated by TM software based on Bayesian Gibbs Sampling Algorithm (**Legarra *et al.*, 2008**). The estimates obtained from Gibbs Sampling were used to solve the corresponding mixed model equations, obtaining the solutions for DO and CI traits using the PEST software (**Groeneveld, 2006**). Therefore, the following single-trait repeatability animal model was used for analyzing DO and CI (Model 3):

$\mathbf{y = Xb + Z_a u_a + Z_p u_p + e}$ (Model 3 Repeatability Single-trait animal model)

Where y = the vector of observed DO and CI trait for the buffalo cow; b = the vector of fixed effects of herd year-season of calving (380 levels), and parity (four levels); u_a = the vector of random additive genetic effects of the buffalo cow; up = the vector of random non-additive permanent effects of the buffalo cow; X, Z_a and Z_p = the incidence matrices relating records to the fixed effects, random additive genetic effects and permanent environment effects, respectively; e = the vector of random residual effects. Data of AFC was analyzed using the same Model 3 after excluding the fixed effect of parity

and the random non-additive permanent effects. Heritabilities for reproduction traits were computed using TM software of Bayesian Gibbs Sampling Algorithm (**Legarra *et al.*, 2008**) as; $\boldsymbol{h^2 = \frac{\sigma^2_a}{\sigma^2_a+\sigma^2_P+\sigma^2_e}}$ for DO and CI traits, while $\boldsymbol{h^2 = \frac{\sigma^2_a}{\sigma^2_a+\sigma^2_e}}$ for AFC trait, where $\boldsymbol{\sigma^2_a}$, $\boldsymbol{\sigma^2_P}$ and $\boldsymbol{\sigma^2_e}$ are previously defined in Model 1.

3.7.3 Animal model for analyzing semen traits

The investigated semen traits were: ejaculate volume (EV), motility of sperms (MS), live sperms (LS), abnormal sperms (AS) and sperms concentration (SC). All the known relationships among animals were considered in analyses of semen traits and the pedigree file comprising a total of 10802 animals with or without records were used. The number of buffalo animals and records belonging to the two studied herds used in data analyses for semen traits are shown in **Table 21**. Data of semen traits were analyzed using single-trait animal model. The variance components of random effects and heritabilities were estimated by TM software of a Bayesian inference Gibbs Sampling Algorithm **(Legarra *et al.*, 2008)**. The estimates obtained from Gibbs sampling were used to solve the corresponding mixed model equations using the PEST software to obtain the solutions of the non-genetic effects and their error variance–covariance matrix **(Groeneveld, 2006)**. Then, the following repeatability single-trait animal model was used:

$$\mathbf{y = Xb + Z_a u_a + Z_p u_p + e \quad (Model\ 4)}$$

Where y = the vector of observed semen trait for the buffalo bull; b = the vector of fixed effects of herd (two levels; IMTC and MM), year-season of semen collection (38 levels) and age of the bull at semen collection (ten levels; 21-30, 31–40, 41–50, 51–60, 61-70, 71-80, 81-95, 96-110, 111-125 and $\geq$126 month of age); u_a = the vector of random additive genetic effect of the bulls; u_p = the vector of random non-additive permanent environmental effects of the buffalo bulls; X, Z_a and Z_p = the incidence matrices relating records to the fixed effects, additive genetic effects and permanent environment effects, respectively; e = the vector of random error.

Heritabilities for semen traits were computed using the TM software of Bayesian Gibbs Sampling Algorithm as reported by **Legarra *et al.* (2008)**: $h^2 = \frac{\sigma^2{}_a}{\sigma^2{}_a+\sigma^2{}_P+\sigma^2{}_e}$, where $\sigma^2{}_a$ = the additive genetic variance of semen traits, $\sigma^2{}_P$ the permanent environmental variance and $\sigma^2{}_e$ = the residual variance.

3.7.4 Animal model for analyzing growth traits

Data of BW, WW and DG were evaluated fitting the fixed effects in the model to avoid over-parameterization. The variance components of random effects and heritabilities were estimated by TM software of a Bayesian Inference Gibbs Sampling Algorithm (**Legarra *et al.*, 2008**). The estimates obtained from Gibbs sampling were used to solve the corresponding mixed model equations, obtaining the generalized least-square means for BW, WW and DG using the PEST software (**Groeneveld, 2006)**. Then, the following single-trait animal model was used:

$$\mathbf{y = Xb + Z_a u_a + Z_c u_c + e} \quad \textbf{(Model 5 Single-trait animal model)}$$

Where **y** = the vector of observed BW or WW or DG of buffalo calves; **b** = the vector of fixed effects of herd-year-season of calf birth (382 levels), sex of calf (males or females), parity order (five levels); $\mathbf{u_a}$ = the vector of random additive genetic effects of the buffalo calves; $\mathbf{u_c}$ = the vector of random common environmental effects; **X,** $\mathbf{Z_a}$ and $\mathbf{Z_c}$= the incidence matrices relating records to the fixed effects, additive genetic effects and random common environmental effects, respectively; **e** = the vector of random residual effects. The heritabilities were estimated for BW, WW and DG traits using the following equation: $\boldsymbol{h^2 = \frac{\sigma^2 a}{\sigma^2 a+ \sigma^2 c+\sigma^2 e}}$, where $\boldsymbol{\sigma^2 a}$ = the additive genetic variance, $\boldsymbol{\sigma^2 c}$ = the maternal common environmental variance and $\boldsymbol{\sigma^2 e}$ = the error variance.

3.8 Predicting breeding values (PBVs) using BLUPF90 program

The predicted breeding values (PBVs), predicted error variance and accuracies of predictions (r_A) for lactation, reproduction, growth and semen traits were estimated using the computer package of BLUPF90 software

(**Misztal *et al.*, 2018; http://nee.ads.uga.edu//wiku///doku.php**). The values of PBV were estimated for 1192 buffaloes for lactation traits of TDMY, TDFY, TDPY and TDSCS using the repeatability animal model mentioned before (**Model 1**), 3285 buffaloes for reproduction traits (**Model 3**), 237 animals for semen traits (**Model 4**) and 10681 animals for growth traits (**Model 5)**. The solutions for the equations of animals were computed from the pedigree file for buffaloes with records and sires and dams without records. The accuracy for PBV (r_A) was defined as the correlation between the true and predicted breeding values and is calculated as described by **Meyer (2004)** as: $r_A = \sqrt{\mathbf{1} - (\boldsymbol{PEV}/\boldsymbol{\sigma}^2\boldsymbol{a})}$, where PEV = the prediction error variance estimated using the elements from the mixed model equations and $\sigma^2{}_a$= the additive genetic variance of the trait.

3.9 Plotting the genetic and phenotypic trends

For lactation traits, the breeding values for a total of 1192 buffaloes with records and without records estimated by BLUPF90 software were used for plotting the genetic trends (**Misztal *et al.*, 2018**). Accordingly, the breeding values for 1192 animals with 7345 lactation records were used to plot the genetic trends by regressing the breeding values for TDMY, TDFY, TDPY and TDSCS on herd-year test day (271 levels). The phenotypic trend for each lactation trait was measured by regressing the phenotypic values of a lactation trait for 7345 lactation records of TDMY, TDFY, TDPY and TDSCS on herd-year-TD (271 levels).

For reproduction traits, the breeding values estimated by BLUPF90 software (**Misztal *et al.*, 2018**) for 7279 reproduction records were used in plotting the genetic trends by regressing the breeding values for AFC, DO and CI on herd-year-season of calving (380 levels). The phenotypic trends were measured as the regression of the phenotypic values for DO, CI and AFC traits on herd-year-season of calving (380 levels).

For semen traits, the phenotypic trends were plotted from 5178 semen ejaculate records by regressing the phenotypic values of EV, MS, LS, AS and

SC on year-season of semen collection (38 levels). The breeding values for semen traits of 237 animals with and without records were estimated by BLUPF90 software software (**Misztal *et al*., 2018**) and the PBV values for bull with records and parents without records were used in plotting the genetic trends by regressing the breeding values of EV, MS, LS, AS and SC on year-season of semen collection.

For growth traits, the breeding values for growth traits of 10681 animals with and without records were estimated by BLUPF90 software (**Misztal *et al*., 2018**) and the PBV values for calves with records and sires and dams without records were used in plotting the genetic trends by regressing the breeding values of body weights and gains on herd-year-season of birth of calves. The phenotypic trends were plotted by regressing the phenotypic values of BW, WW and DG for records of 8229 calves for BW, 8203 calves for WW and 8181 calves for DG on herd-year-season of birth of calves (382 levels).

3.10 Molecular genetic analyses

3.10.1 Animals and records used in molecular analyses

Concerning lactation traits, a total of 200 blood samples were collected from buffalo cows randomly selected from the studied buffalo herds for genotyping. Out of these samples, a total of 103 animals (about 52 % of the total blood samples) were successfully genotyped using PCR-RFLP. For lactation traits, a total of 103 lactating buffalo cows (with 1029 lactation records) from NG and NK herds were used for the molecular analyses of the candidate genes (*PRL*, *DGAT1*, *FSHR* and *GH*) associated with these traits. For reproduction traits, a total of 103 buffalo cows (with 453 reproduction records) from NG, NK and EG herds were used for the molecular analyses of the candidate genes (*PRL*, *FSHR* and *GH*) associated with these traits.

For the molecular genetic analyses of semen traits, a total of 86 blood samples were collected from buffalo bulls randomly selected of the studied buffalo herd for genotyping. Out of these samples, a total of 71 animals (about 83% of the total blood samples) were successfully genotyped using

PCR-RFLP. For semen traits, a total of 71 bulls (with 713 ejaculate records) from MM herd was used for the molecular analyses of the candidate genes (*FSHR* and *GH*) associated with semen traits.

Regarding growth traits, blood samples from 286 buffalo calves (200 female and 86 male) were collected randomly from of the studied buffalo herds for genotyping. A total of 174 genotyped calves (about 61% of the total blood samples) from NG, NK and EG herds has been used for the molecular analyses of the candidate genes (*GH, PRL* and *FSHR*) associated with growth traits. All available relationships among calves were considered in molecular data analyses.

3.10.2 Blood sampling and DNA extraction

For DNA extraction and amplification, blood samples were collected from the jugular veins of the investigated animals in vacutainer tubes containing EDTA. All the samples were labeled, stored in an ice box and transferred to the laboratory and stored at -20ºC to be used in further processing. Genomic DNA was extracted from leukocytes using the QIAamp® Whole Blood Genomic DNA purity Kit (QIAGEN, Hilden, Germany). An amount of 20 μl of proteinase K solution was added to 200 μl of whole blood in 2 ml Eppendorf tube and mixed by overtaxing; then 200 μl of lysis solution was added and mixed thoroughly by pipetting to obtain a uniform suspension. The sample was incubated at 56^{o}C for 10 minutes using a shaking water bath until the cells were completely lysed. 200 μl ethanol (96–100%) was added to the sample and remixed by pulse-vortex for 15 seconds. The prepared mixture was transferred to the spin column, centrifuged at 6000 rpm for one minute at room temperature and then the collection tube containing the flow-through solution was removed. The spin column was placed into a new 2 *ml* collection tube; then 500 μl of wash buffer AW1 was added and centrifuged at 8000 rpm for one minute at room temperature. The flow-through solution was discarded and the column placed back into the collection tube. A 500 μl of wash buffer AW2 was added to the column and centrifuged at 14000 *rpm* for three minutes at room temperature.

The collection tube was emptied and the purification column placed back into the tube and centrifuged at full speed for one minute. This step helps to eliminate the chance of possible Buffer AW2 carryover. The collection tube containing the flow-through solution was discarded, transferring the column to a sterile 1.5 *ml* micro centrifuge tube. A 200 *µl* Buffer AE or distilled water was added to the center of the column membrane to elute the genomic DNA, incubated for two minutes and centrifuged at 8000 *rpm* for one minute at room temperature (15–25°C). Genomic DNA was stored at -20^{o}C. Then, high quality purified and concentrated DNA products were obtained to be used directly in a variety of downstream applications.

3.10.3 Amplification by polymerase chain reaction (PCR)

The PCR technique was used to amplify *PRL*, *DGAT1*, *FSHR* and *GH* genes. PCR product processing was performed in 25 *µl* reaction mixtures, containing 1.5 *mM* $MgCl_2$, 200 *µM* dNTP mix, 5 *pmol* of each primer, 10 × PCR buffer, 1 U Taq DNA polymerase and 100 *ng* of genomic DNA. The primers used in the amplification process are given in **Table 23**.

A 678 *bp* fragment of *PRL* gene was amplified using the following primer set forward 5′ -AGGTTAGGAGGATAG-3′ and reverse 5′ -TTAGTCAAGTTAGATACCG-3′ (**Hasanain *et al.*, 2017**). The thermal cycling conditions were composed of a pre-denaturation step at 95°C for three minutes, followed by 35 cycles at 95°C for one minute, 50.6°C for 60 seconds, extension at 72°C for one minute and the final extension at 72°C for five minutes.

A 411 *bp* fragment of Diacylglycerol acyltransferase gene (DGAT1) was amplified using a primer forward 5´-GCACCATCCTCTTCCTCAAG-3´and reverse 5´-GGAAGCGCTTTCGG ATG-3´ (**Özdil and Ilhan, 2012**). The thermal cycling conditions were composed of a pre-denaturation step at 95°C for 15 minutes, followed by 35 cycles of denaturation at 94°C for one minute, annealing at 60˚C for one minute, elongation at 72°C for one minute and then final extension at 72˚C for ten minutes.

A 306 *bp* fragment of *FSHR* gene was amplified using the following primer set forward 5´-CTGCCTCCCTCAAGGTGCCCCTC-3′ and reverse 5'-AGTTCTTGGCTAAATGTCTTAGGGGG -3' (**Fouda *et al.*, 2021**). The PCR reaction was conducted as following: PCR tubes containing the mixture were subjected to five minutes at 95 ºC for initial denaturation, 30 cycles of amplification (denaturation at 95 ºC for thirty seconds, annealing at 60 ºC for thirty seconds and extension at 72 ºC for thirty seconds) and final extension at 72 ºC for eight minutes.

A 211 *bp* fragment of *GH* gene was amplified using the following primer set forward 5´- GCTGCTC CTGAGGGCCCTTC - 3´ and reverse 5'-CATGACCCTCAGGTACGTCTC CG -3' (**Konca and Akyüz, 2017**). The thermal cycling conditions were composed of a pre-denaturation step at 94°C for five minutes, followed by 30 cycles of denaturation at 94 ºC for one minute, annealing at 62ºC for one minute and extension at 72ºC for one minute and then final extension at 72˚C for five minutes.

Aliquots of 10 *µl* of the PCR amplicons for *PRL, DGAT1*, *FSHR* and *GH* genes were electrophoresed using 2 % Ethidium Bromide agarose gel at constant voltage of 100 for 30 minutes, then visualized under UV light with a Gel Doc 1000 system (Bio-Rad).

3.10.4 Digestion and genotyping of *PRL, DGAT1, FSHR* and *GH* genes using PCR-RFLP technique

To characterize *PRL, DGAT1, FSHR* and *GH* genes according to their restriction pattern, the PCR product of each gene was digested with the proper restriction enzyme, as specified in **Table 23**. Each enzymatic reaction consisted of a 25 *µl* mix including 0.5 *µl* (10u/*µl*) of restriction enzyme (Fermentas), 2.5*µl* of 10x NE Buffer, 5 *µl* of PCR product, 0.1 *mg/ml* acetylated Bovine serum albumin (BSA), and 16.75 *µl* of sterile dH2O. The digested fragments were visualized by electrophoresis on 2.5 % agarose gel at 120 V in 1xTAE. The 250 *bp* DNA step ladder (Promega) was included in each run.

Table 23. Primer sequence and PCR-RFLP assay conditions for genotyping SNPs of *PRL, DGAT1 FSHR* and *GH*, genes

Gene	CN+	Primer sequences (forward/reverse)	PCR Product size (bp)	Annealing temp (ºC per time, s)	Restriction Enzyme
PRL	2	5′ -AGGTTAGGAGGATAG-3′ 5′ -TTAGTCAAGTTAGATACCG-3′	678	50.5/60	*XbaI*
DGAT1	15	5′ -GCACCATCCTCTTCCTCAAG -3´ 5´-GGAAGCGCTTTCGGATG -3´	411	60/60	*AluI*
FSHR	12	5´CTGCCTCCCTCAAGGTGCCCCT C-3′ 5'AGTTCTTGGCTAAATGTCTTAGG GGG-3'	306	60/30	*AluI*
GH	3	5´-GCTGCTCCTGAGGGCCCTTC -3´ 5'CATGACCCTCAGGTACGTCTCC G -3'	211	62/60	*AluI*

+CN= Chromosome number.

After electrophoresis, the gel was stained with ethidium bromide 0.5 µg/ml. Fragments were visualized a UV transilluminator and the images were digitalized by the Gel Doc^{TM}XR^{+}(BIO-RAD) gel documentation system. The PCR-RFLP technique was used in genotyping the SNP genotypes located in the promoter regions of these genes. Also, PCR-RFLP technique and *XbaI* and *AluI* restriction enzymes were used to detect the molecular associations between SNP genotypes of *PRL, DGAT1, FSHR* and *GH* candidate genes and lactation , reproduction, semen and growth traits in the Egyptian buffalo.

3.10.5 Molecular parameters to characterize *PRL, DGAT1, FSHR* and *GH* genes

From the 286 collected blood samples, a total of 101 buffaloes were successfully genotyped for *PRL* and *DGAT1* genes, 98 buffalo cow and 71 buffalo bulls for *FSHR* gene and 103 buffalo cow and 71 buffalo bulls for *GH* gene. The genetic diversity of *PRL, DGAT1*, *FSHR* and *GH* genes were assessed in each herd separately and across all herds by calculating the effective number of alleles (*Ne*), Chi-square values for Hardy-Weinberg equilibrium (*HWE*) and the observed (*Ho*) and expected (*He*)

heterozygosities using GENALEX software version 6.5 (**Peakall and Smouse, 2006**). The following equations were used in estimating the previous parameters:

$$Ne = \frac{1}{\sum_{i=1}^{n} p_i^2} \qquad Ho = \frac{No.\ of\ heterozygosity}{n} \qquad He = 1 - \sum_{i=1}^{n} p_i^2$$

The polymorphism information content (PIC) was calculated using CERVUS software version 3 (**Kalinowski *et al.*, 2007**) as:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \cdot \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where *Pi* = the frequency of the i^{th} allele, *Pj* = the frequency of the j^{th} allele and n = the number of alleles.

3.10.6 Models for detecting the polymorphic associations between *PRL* or *FSHR* or *GH* genes and the studied traits

For association analysis, the number of lactation records gathered in NG and NK herds as well as the number of reproduction records obtained in NG, NK and EG studied buffaloes herds were presented in **Table 24**. The estimates obtained from Bayesian Gibbs Sampling Algorithm (Models 1, 3, 4 and 5) were used to solve the corresponding mixed model equations and obtain the generalized least-square means (GLSM) for lactation, reproduction, semen and growth traits of different genotypes using the PEST software (**Groeneveld, 2006**).

To detect the molecular associations between *PRL* or *FSHR* or *GH* gene with lactation traits (TDMY, TDFY, TDPY and TDSCS), Model 1 previously defined was used after adding the fixed effects of SNP genotypes (AA and GG genotypes for *PRL* gene; GG, GC and CC genotypes for *FSHR* gene; TC and CC genotypes for *GH* gene).

Table 24. Number of records used in molecular association analyses for lactation, reproduction, semen and growth traits in buffalo herds studied

Herd and gene	NG herd	NK herd	EG herd	MM herd	All herds
***PRL* gene analyses:**					
Lactation traits	324	705	--	--	1029
Reproduction traits	143	210	100	--	453
Growth traits	33	45	23	--	101
***FSHR* gene analyses:**					
Lactation traits	435	767	--	--	1202
Reproduction traits	151	196	84	--	431
Semen traits	--	--	--	713	713
Growth traits	47	101	24	--	172
***GH* gene analyses:**					
Lactation traits	324	705	--	--	1029
Reproduction traits	143	224	93	--	460
Semen traits	--	--	--	638	638
Growth traits	51	70	53	--	174

To detect the molecular associations between *PRL* or *FSHR* or *GH* gene with reproduction traits (AFC, CI and DO), Model 3 previously defined was used after adding the fixed effects of SNP genotypes (AA and GG genotypes for *PRL* gene; GG, GC and CC genotypes for *FSHR* gene; TC and CC genotypes for *GH* gene).

To detect the molecular associations between *FSHR* or *GH* gene with semen traits (EV, MS, LS, AS and SC), Model 4 previously defined was used after adding the fixed effects of SNP genotypes (GG, GC and CC genotypes for *FSHR* gene; TC and CC genotyps for *GH* gene).

To detect the molecular associations between *PRL* or *FSHR* or *GH* gene with growth traits (BW, WW and DG), Model 5 previously defined was used after adding the fixed effects of SNP genotypes (AA and GG genotypes for *PRL* gene; GG, GC and CC genotypes for *FSHR* gene; TC and CC genotypes for *GH* gene).

4. RESULTS AND DISCUSSION

4.1 Lactation traits in Egyptian buffalo

4.1.1 Descriptive statistics for lactation traits

The generalized least square means (GLSM), standard deviations (SD), standard errors (SE), minimum and maximum values and coefficients of variation (CV %) for lactation traits are shown in **Table (25)**. The GLSM for lactation traits were 5.76, 7.49, 7.79, 7.34, 6.72, 5.98, 5.28, 4.65 and 4.41 kg for TDMY, 0.362, 0.485, 0.512, 0.485, 0.449, 0.402, 0.354, 0.316 and 0.313 kg for TDFY, 0.229, 0.288, 0.301, 0.282, 0.261, 0.233, 0.210, 0.185 and 0.177 kg for TDPY and 2.01, 2.01, 2.02, 2.01, 2.02, 2.05, 2.10, 2.05 and 1.92 $\log^{10}$ for TDSCS of the consecutive TD monthly traits, respectively. These TD estimates were in accordance with those obtained by **El-Bramony *et al*. (2004, 2017)** and **Amin *et al*. (2015)** on Egyptian buffalo, while were greater than those obtained by **Aspilcueta-Borquis *et al*. (2012)** on Murrah buffalo, by **Sahoo *et al*. (2014)** on Indian buffalo and by **Madad *et al*. (2013)** on Iranian buffalo.

Wide ranges between minimum and maximum values for TD lactation traits were detected, ranging from 1.5 to 20 kg for TDMY, 0.1 to 1.7 kg for TDFY and 0.1 to 0.8 kg for TDPY and 1.0 to 3.6 $\log^{10}$ for TDSCS (**Table 25**). In Egyptian buffalo, **Amin *et al*. (2015)** and **El-Bramony *et al*. (2017)** reported that the ranges between minimum and maximum values for TDMY were 5.14 to 8.51 kg. The coefficients of variation (CV%) values for lactation traits were mostly moderate or high but decreased with the advancement of TD (**Table 25**) and ranged from 31 to 52 % for TDMY, TDFY and TDPY, while they were 22 to 24% for TDSCS. The large coefficients of variation for lactation traits represent good opportunities for selection and possible genetic improvement for these traits. Similarly, coefficients of variation for lactation traits in Egyptian buffalo were mostly moderate or high, ranging from 19.6 to 41.57% for TDMY, 23.9 to 39.85% for TDFY and 21.2 to 40.87% for TDPY as reported by **Amin *et al*. (2015)**

on Egyptian buffalo, **Tonhati *et al*. (2008)** and **Aspilcueta-Borquis *et al*. (2012)** on Murrah buffalo and **Madad *et al*. (2013)** on Iranian buffalo

Table 25. The generalized least square means (GLSM), standard deviations (SD), standard errors (SE), minimum and maximum values and coefficients of variation (CV) for test-day (TD) lactation traits in Egyptian buffalo

Lactation traits (N= 7345 records)	GLSM	SD	SE	Minimum value	Maximum value	CV
TD1 at 4 days in milk (N= 941records):						
TDMY (*kg*)	5.76	2.63	0.086	1.5	18.0	46
TDFY (kg)	0.362	0.188	0.006	0.1	1.7	52
TDPY (kg)	0.229	0.106	0.003	0.1	0.7	46
TDSCS (log^{10})	2.01	0.479	0.016	1.0	3.4	24
TD2 at 30 days in milk (N= 1055 records):						
TDMY (kg)	7.49	2.99	0.092	2.0	20.0	40
TDFY (kg)	0.485	0.225	0.007	0.1	1.4	46
TDPY (kg)	0.288	0.119	0.004	0.1	0.8	41
TDSCS (log^{10})	2.01	0.489	0.015	1.0	3.3	24
TD3 at 60 days in milk (N= 1094 records):						
TDMY (kg)	7.79	2.76	0.083	2.0	18.0	35
TDFY (kg)	0.512	0.209	0.006	0.1	1.4	41
TDPY (kg)	0.301	0.112	0.003	0.1	0.8	37
TDSCS (log^{10})	2.02	0.487	0.015	1.0	3.2	24
TD4 at 90 days in milk (N= 1120 records):						
TDMY (kg)	7.34	2.73	0.082	2.0	18.0	37
TDFY (kg)	0.485	0.201	0.006	0.1	1.4	41
TDPY (kg)	0.282	0.112	0.003	0.1	0.8	40
TDSCS (log^{10})	2.01	0.485	0.014	1.0	3.6	24
TD5 at 120 days in milk (N= 1051 records):						
TDMY (kg)	6.72	2.48	0.077	2.0	17.0	37
TDFY (kg)	0.449	0.185	0.006	0.1	1.3	41
TDPY (kg)	0.261	0.101	0.003	0.1	0.7	39
TDSCS (log^{10})	2.02	0.484	0.015	1.0	3.3	24
TD6 at 150 days in milk (N= 922 records):						
TDMY (kg)	5.98	2.11	0.069	1.5	15.0	35
TDFY (kg)	0.402	0.163	0.005	0.1	1.1	41
TDPY (kg)	0.233	0.084	0.003	0.1	0.6	36
TDSCS (log^{10})	2.05	0.456	0.015	1.0	3.4	22

Table 25. Cont.

Lactation traits (N= 7345 records)	GLSM	SD	SE	Minimum value	Maximum value	CV
TD7 at 180 days in milk (N = 652 records):						
TDMY (kg)	5.28	1.84	0.072	1.5	15.0	35
TDFY (kg)	0.354	0.150	0.006	0.1	1.3	42
TDPY (kg)	0.210	0.076	0.003	0.1	0.6	36
TDSCS (log^{10})	2.10	0.456	0.018	1.0	3.2	22
TD8 at 210 days in milk (N= 354 records):						
TDMY (kg)	4.65	1.45	0.077	2.0	12.5	31
TDFY (kg)	0.316	0.131	0.007	0.1	0.9	41
TDPY (kg)	0.185	0.062	0.003	0.1	0.5	33
TDSCS (log^{10})	2.05	0.455	0.024	1.1	2.9	22
TD9 at 240 days in milk (N= 156 records):						
TDMY (kg)	4.41	1.42	0.114	2.0	8.5	32
TDFY (kg)	0.313	0.120	0.010	0.1	0.6	38
TDPY (kg)	0.177	0.063	0.005	0.1	0.4	36
TDSCS (log^{10})	1.92	0.427	0.034	1.1	3.4	22

TDMY= Test-day milk yield, TDFY= Test-day fat yield, TDPY= Test-day protein yield and TDSCS (log^{10}) = Test-day somatic cell score.

4.1.2 Heritability estimates and permanent environmental effects for lactation traits

Heritability values estimated by repeatability single-trait animal model for lactation traits were mostly moderate ranging from 0.05 to 0.40 for TDMY, 0.05 to 0.45 for TDFY, 0.06 to 0.44 for TDPY and 0.03 to 0.39 for TDSCS (**Table 26**). Thus, selection for lactation traits in Egyptian buffalo could be performed efficiently. These estimates were within the range of those heritability values estimated by animal model in other studies on Egyptian buffalo (**Ibrahim *et al.*, 2012**; **El-Bramony *et al.*, 2017**), Brazilian Murrah buffalo (**Tonhati *et al.*, 2008**; **De Camargo *et al.*, 2015**) and Indian Murrah buffalo (**Sahoo *et al.*, 2014; Singh *et al.*, 2016**). The proportions of permanent environmental effects (p^2) estimated by animal model for lactation traits were moderate, ranging from 0.10 to 0.31 for TDMY, 0.06 to 0.29 for TDFY, 0.09 to 0.25 for TDPY and from 0.07 to 0.22 for TDSCS (**Table 26**), *i.e.* the lactation traits of buffalo become sensitive to the environmental and

management changes throughout the lactation period. **El-Bramony *et al.* (2017)** reported that the p^2 estimated by animal model for lactation traits were high and ranged from 0.56 to 0.74 for TDMY, 0.53 to 0.69 for TDFY and 0.51 to 0.70 for TDPY.

Heritability values estimated by RRM for lactation traits were mostly low at the beginning of lactation, increased gradually to reach the highest value then decreased gradually to reach the lowest value towards the end of lactation, the estimates ranged from 0.04 to 0.25 for TDMY, 0.05 to 0.18 for TDFY, 0.03 to 0.23 for TDPY and 0.07 to 0.57 for TDSCS (**Table 26**). Similarly, **Amin *et al.* (2015)** reported definite trend for heritability values estimated by RRM for milk yield in Egyptian buffalo to be low at the beginning of the TD (0.05 to 0.28) and gradually increased to reach the highest value at the fourth TD (0.28 and 0.31), then the estimates decreased gradually until reaching the lowest value at the tenth TD (0.06 to 0.10). **Aspilcueta-Borquis *et al.* (2012)** found that heritability estimates in Brazilian Murrah buffalo estimated by RRM were 0.16 to 0.29, 0.20 to 0.30 and 0.18 to 0.27 for TDMY, TDFY and TDPY, respectively. The proportions of p^2 estimated by RRM for milk, fat and protein yields were mostly low or moderate, ranging from 0.05 to 0.09, 0.17 to 0.21, 0.26 to 0.28, 0.28 to 0.31, 0.27 to 0.31, 0.23 to 0.27, 0.18 to 0.21, 0.09 to 0.16 and 0.02 to 0.12 for the consecutive TD number between one and nine (**Table 26**), while the estimates of p^2 for TDSCS were mostly high, ranging from 0.18 to 0.59. **El-Bramony *et al.* (2017)** reported that p^2 estimated by RRM ranged from 0.09 to 0.31 for TDMY, 0.02 to 0.31 for TDFY, 0.05 to 0.28 for TDPY and 0.18 to 0.59 for TDSCS. **Aspilcueta-Borquis *et al.* (2012)** reported that the p^2 estimated by RRM in Murrah buffalo were moderate or high, ranging from 0.35 to 0.45 for TDMY, 0.30 to 0.52 for TDFY and 0.40 to 0.45 for TDPY. Recently, **Ranjan *et al.* (2023)** in Murrah buffalo showed that the p^2 estimated by RRM for TDMY were high and ranged from 0.21 to 0.85.

Table 26. Heritability estimates (h^2) and proportions of permanent environmental effects (p^2) and random error effects (e^2) for test-day (TD) lactation traits in Egyptian buffalo

Lactation traits (N= 7345 record)	Animal Model			Random Regression Model		
	h^2±SE	p^2±SE	e^2±SE	h^2	p^2	e^2
TD1 at 4 days in milk (N= 941 record):						
TDMY (*kg*)	0.07 ±0.06	0.24±0.06	0.68±0.05	0.22	0.09	0.69
TDFY (kg)	0.07 ±0.05	0.21±0.06	0.72±0.05	0.16	0.09	0.75
TDPY (kg)	0.08±0.06	0.21 ±0.07	0.70±0.05	0.23	0.05	0.72
TDSCS (log^{10})	0.07±0.05	0.07±0.04	0.8 ±0.04	0.07	0.58	0.35
TD2 at 30 days in milk (N= 1055 record):						
TDMY (kg)	0.09 ±0.06	0.17 ±0.06	0.74±0.05	0.25	0.21	0.54
TDFY (kg)	0.05±0.04	0.10±0.05	0.84±0.04	0.17	0.21	0.62
TDPY (kg)	0.08±0.06	0.11±0.05	0.80±0.05	0.22	0.17	0.60
TDSCS (log^{10})	0.06±0.05	0.18±0.05	0.76±0.05	0.24	0.51	0.25
TD3 at 60 days in milk (N= 1094 record):						
TDMY (kg)	0.20±0.09	0.24±0.08	0.56 ±0.04	0.24	0.28	0.48
TDFY (kg)	0.10±0.07	0.18±0.07	0.71±0.05	0.15	0.28	0.57
TDPY (kg)	0.14±0.08	0.15±0.07	0.71±0.05	0.19	0.26	0.55
TDSCS (log^{10})	0.06±0.04	0.16±0.05	0.79±0.04	0.23	0.55	0.21
TD4 at 90 days in milk (N= 1120 record):						
TDMY (kg)	0.05±0.05	0.31±0.06	0.63±0.04	0.20	0.31	0.48
TDFY (kg)	0.05±0.05	0.24±0.06	0.71±0.05	0.12	0.31	0.57
TDPY (kg)	0.06±0.06	0.25±0.06	0.68 ±0.05	0.16	0.28	0.55
TDSCS (log^{10})	0.10±0.08	0.18±0.06	0.71±0.05	0.25	0.54	0.21
TD5 at 120 days in milk (N= 1051 record):						
TDMY (kg)	0.09±0.06	0.30±0.06	0.61±0.04	0.16	0.31	0.53
TDFY (kg)	0.10±0.07	0.20± 0.06	0.69±0.05	0.08	0.30	0.62
TDPY (kg)	0.11±0.08	0.19±0.07	0.69±0.04	0.13	0.27	0.60
TDSCS (log^{10})	0.10±0.06	0.13±0.06	0.77±0.05	0.18	0.59	0.23
TD6 at 150 days in milk (N= 922 record):						
TDMY (kg)	0.08 ± 0.06	0.14 ±0.06	0.78±0.05	0.11	0.27	0.62
TDFY (kg)	0.05±0.04	0.09±0.05	0.86±0.05	0.05	0.26	0.69
TDPY (kg)	0.08 ±0.06	0.11 ±0.06	0.80±0.05	0.09	0.23	0.68
TDSCS (log^{10})	0.03±0.03	0.14 ±0.05	0.82± 0.05	0.25	0.49	0.26

Table 26. Cont.

Lactation traits (N= 7345 record)	Animal Model			Random Regression Model		
	h^2±SE	p^2±SE	e^2±SE	h^2	p^2	e^2
TD7 at 180 days in milk (N = 652 record):						
TDMY (kg)	0.09±0.06	0.10±0.06	0.82±0.07	0.06	0.21	0.72
TDFY (kg)	0.06±0.05	0.06±0.04	0.88±0.05	0.05	0.18	0.77
TDPY (kg)	0.08±0.06	0.09±0.06	0.83±0.07	0.06	0.18	0.76
TDSCS (log^{10})	0.11±0.08	0.21±0.09	0.68±0.07	0.25	0.46	0.29
TD8 at 210 days in milk (N= 354 record):						
TDMY (kg)	0.18±0.13	0.20±0.11	0.62±0.09	0.04	0.16	0.80
TDFY (kg)	0.15±0.11	0.13±0.09	0.72±0.11	0.09	0.09	0.82
TDPY (kg)	0.19±0.13	0.21±0.11	0.60 ±0.09	0.03	0.15	0.82
TDSCS (log^{10})	0.13±0.11	0.13±0.11	0.71±0.12	0.40	0.31	0.29
TD9 at 240 days in milk (N= 156 record):						
TDMY (kg)	0.40±0.23	0.24±0.19	0.35±0.20	0.04	0.12	0.84
TDFY (kg)	0.45 ±0.25	0.29±0.22	0.24±0.19	0.18	0.02	0.80
TDPY (kg)	0.44±0.24	0.25 ±0.20	0.30±0.20	0.03	0.11	0.85
TDSCS (log^{10})	0.39±0.24	0.22 ±0.19	0.38±0.22	0.57	0.18	0.24

SE= standerd; TDMY= Test-day milk yield, TDFY= Test-day fat yield, TDPY= Test-day protein yield and TDSCS = Test-day somatic cell score

4.1.3 Predicted breeding values (PBV) for lactation traits

Estimates of minimum and maximum PBVs and their accuracy of predictions (r_A) and ranges for TDMY, TDFY, TDPY and TDSCS are given in **Table (27)**. The ranges in PBVs were moderate or high, being -2.01 to 3.4 *kg* for TDMY, -0.358 to 0.521 *kg* for TDFY, -0.053 to 0.095 *kg* for TDPY and -0.183 to 0.313 log^{10} for TDSCS. The ranges in PBVs decreased up to TD4 and then increased till the end of lactation TD9. The reviewed estimates of PBVs on Egyptian buffalo were moderate or high, ranging from -1548 to 2954 kg for total milk yield, -85 to 93 kg for total fat yield, -47 to 44 kg for total protein yield and -1.16 to 8.03 (log^{10}) for somatic cell score (**Khattab *et al*., 2003; El-Arian *et al*., 2012; Shalaby *et al*., 2016; Ahmad *et al*., 2017; Abo-Gamil *et al*., 2017; EL-Hedainy *et al*., 2020**).

Table 27. Minimum and maximum predicted breeding values (PBVs), standard errors (SE) and accuracy of predictions (r_A) for test-day (TD) lactation traits in Egyptian buffalo estimated by single-trait Animal Model using BLUPF90 software

Lactation traits (N= 1192 animal with 7345 records)	Minimum PBV			Maximum PBV			Range in PBV	Positive PBV (%)
	PBV	SE	r_A	PBV	SE	r_A		
TD1 at 4 days in milk (N= 1031 animals):								
TDMY (*kg*)	-0.765	0.427	0.501	1.301	0.428	0.498	2.06	52
TDFY (kg)	-0.058	0.034	0.489	0.088	0.034	0.471	0.147	53
TDPY (kg)	-0.032	0.019	0.494	0.041	0.019	0.504	0.073	50
TDSCS (log^{10})	-0.087	0.086	0.408	0.110	0.083	0.467	0.197	63
TD2 at 30 days in milk (N= 1096 animals):								
TDMY (kg)	-1.156	0.532	0.587	1.815	0.512	0.627	2.97	53
TDFY (kg)	-0.059	0.036	0.574	0.085	0.038	0.514	0.145	52
TDPY (kg)	-0.026	0.022	0.406	0.049	0.024	0.548	0.075	56
TDSCS (log^{10})	-0.129	0.066	0.659	0.145	0.078	0.469	0.275	55
TD3 at 60 days in milk (N= 1109 animals):								
TDMY (kg)	-2.005	0.713	0.669	3.419	0.671	0.715	5.42	53
TDFY (kg)	-0.358	0.055	0.315	0.521	0.054	0.376	0.878	57
TDPY (kg)	-0.052	0.027	0.599	0.095	0.027	0.605	0.147	52
TDSCS (log^{10})	-0.091	0.077	0.422	0.147	0.081	0.333	0.235	57
TD4 at 90 days in milk (N= 1121 animals):								
TDMY (kg)	-1.024	0.426	0.562	1.638	0.450	0.488	2.66	54
TDFY (kg)	-0.058	0.033	0.535	0.064	0.036	0.434	0.123	57
TDPY (kg)	-0.040	0.019	0.569	0.051	0.020	0.477	0.091	53
TDSCS (log^{10})	-0.151	0.103	0.475	0.212	0.089	0.644	0.363	65
TD5 at 120 days in milk (N= 1090 animals):								
TDMY (kg)	-0.952	0.515	0.717	1.928	0.502	0.709	2.88	58
TDFY (kg)	-0.072	0.045	0.557	0.126	0.043	0.618	0.199	59
TDPY (kg)	-0.044	0.024	0.567	0.089	0.024	0.057	0.132	60
TDSCS (log^{10})	-0.123	0.098	0.529	0.185	0.094	0.454	0.308	60
TD6 at 150 days in milk (N= 997 animals):								
TDMY (kg)	-0.505	0.434	0.507	0.712	0.406	0.589	1.22	60
TDFY (kg)	-0.019	0.031	0.429	0.037	0.029	0.506	0.056	60
TDPY (kg)	-0.022	0.019	0.522	0.042	0.017	0.607	0.064	64
TDSCS (log^{10})	-0.048	0.052	0.435	0.056	0.053	0.369	0.104	58
TD7 at 180 days in milk (N = 836 animals):								
TDMY (kg)	-0.338	0.417	0.402	1.249	0.368	0.590	1.587	72
TDFY (kg)	-0.021	0.031	0.984	0.081	0.028	0.986	0.102	73
TDPY (kg)	-0.012	0.017	0.493	0.043	0.016	0.575	0.055	74
TDSCS (log^{10})	-0.144	0.088	0.496	0.162	0.089	0.483	0.306	66

Table 27. Cont.

Lactation traits (N= 1192 animal with 7345 records)	Minimum PBV			Maximum PBV			Range in PBV	Positive PBV (%)
	PBV	SE	r_A	PBV	SE	r_A		
TD8 at 210 days in milk (N= 562 animals):								
TDMY (kg)	-0.541	0.478	0.520	0.992	0.449	0.596	1.533	73
TDFY (kg)	-0.046	0.039	0.535	0.044	0.041	0.513	0.089	74
TDPY (kg)	-0.025	0.021	0.534	0.041	0.021	0.556	0.066	77
TDSCS (log^{10})	-0.137	0.101	0.524	0.114	0.108	0.392	0.251	74
TD9 at 240 days in milk (N= 241 animals):								
TDMY (kg)	-0.934	0.656	0.727	1.415	0.645	0.738	2.35	74
TDFY (kg)	-0.063	0.039	0.887	0.163	0.049	0.809	0.226	74
TDPY (kg)	-0.053	0.027	0.778	0.057	0.027	0.778	0.109	77
TDSCS (log^{10})	-0183	0.173	0.724	0.313	0.173	0.723	0.497	68

TDMY= Test-day milk yield, TDFY= Test-day fat yield, TDPY= Test-day protein yield and TDSCS= Test-day somatic cell score.

The percentages of experimental animals (buffaloes, sires and dams) having positive PBVs for TDMY, TDFY, TDPY and TDSCS were more than 50 % and ranged from 52 to 74 % for TDMY and TDFY, 50 to 77 % for TDPY and 55 to 74 % for TDSCS (**Table 27**). The high positive PBVs for such lactation traits reveal a good opportunity for genetic improvement of total productive merit of buffaloes when including these traits in a selection scheme.

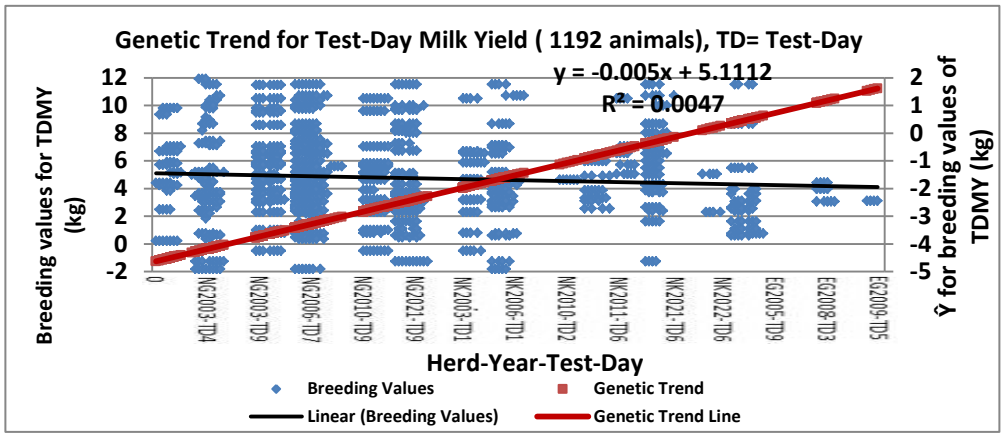
The accuracies of prediction (r_A) for minimum and maximum estimates of PBVs were moderate or high in most TD milk yields and compositions, ranging from 0.315 to 0.986 for lactation traits (**Table 27**). These high accuracies may be because heritability estimates were highly associated with more available pedigree information for all animals (**Korhonen, 1996**). Such high accuracies in PBVs obtained in the present study suggest that selection plans to be used in future generations would lead to sustainable genetic improvement for lactation traits in Egyptian buffalo.

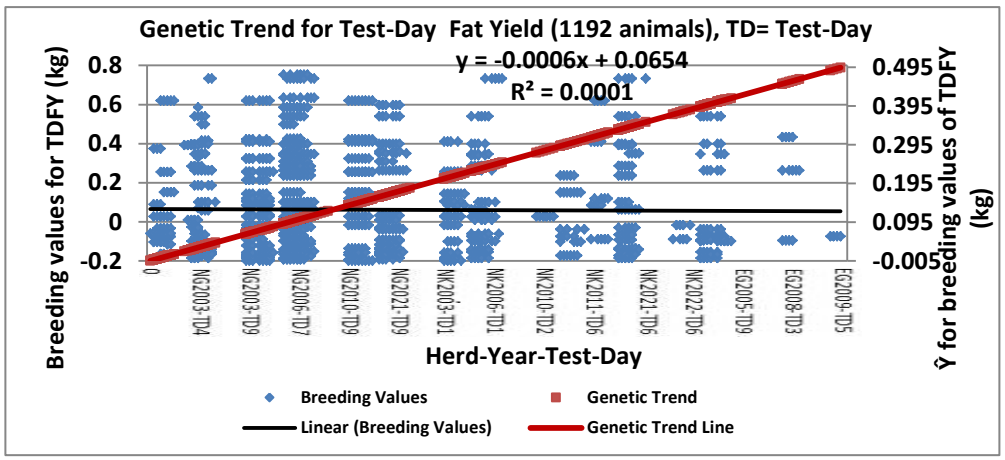
4.1.4 Genetic and phenotypic trends for lactation traits

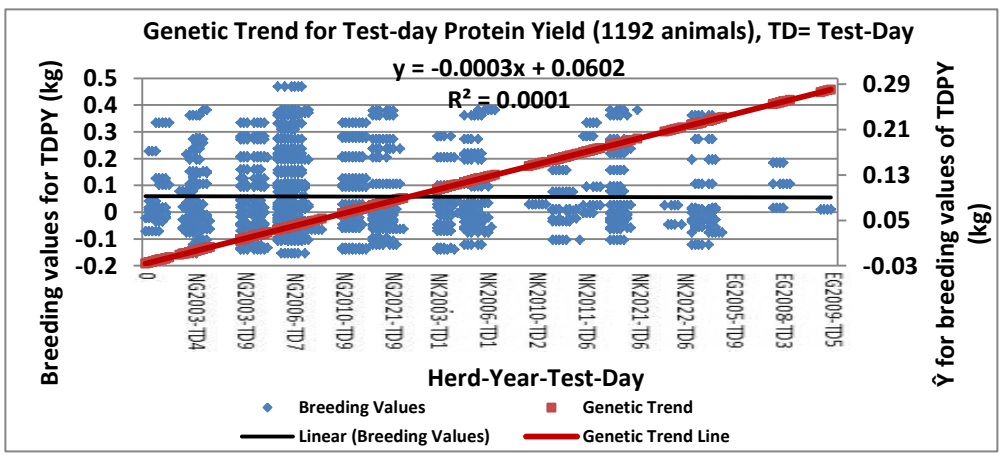
The genetic trends plotted for TDMY, TDFY, TDPY and TDSCS across the years from 2003 to 2023 are shown in **Figure 1.** The regression line of PBVs on TD lactation traits of 1192 animals (buffaloes with records and sires and dams without records) indicated favorable increase in genetic trend line of milk, fat and protein yields associated with favorable decrease in the genetic trend line for TDSCS as herd-year of TD advanced. The ranges of genetic trends for TD lactation traits were favorably increased from -4.63 to 1.61 kg for TDMY, -5.0 to 495 g for TDFY and -26 to 280 g for TDPY, along with favorable decrease of 1.37 to 1.19 $\log^{10}$ in the genetic trend of TDSCS over time of lactation. Such wide ranges of genetic trends reflect an appropriate culling and replacement practices performed in these herds. Also, the positive genetic trends for all lactation traits were resulting from the selection program applied for these traits in the experimental herds studied. The slight increase in genetic trend registered over 20 years of recording activity in the present study could be explained depending on the following facts: 1) progeny testing of selection could not practice in the proper direction for lactation traits and it was not performed on a large scale due to the difficulties to use artificial insemination in buffalo herds efficiently, 2) selection was not much effective to be in the desired changes over 20 years due to natural insemination was applied and low management practices for the improvements in lactation performance, 3) the size of the lactating buffaloes in the herds was small, 4) inbreeding was practiced in few cases, 5) sometimes there are problems in recording milk production quantities and components, and 6) In recent years, the breeding strategy relied on only few proven sires due to challenging of economic conditions and a lack of funding, which led to the exclusion of many proven sires.

The phenotypic trends plotted for TDMY, TDFY, TDPY and TDSCS throughout the experimental period of 21 years (2003 to 2023) showed an apparent deteriorating trend (Figure 2), indicating that the change in environmental situations along with inefficient management strategy during the last 20 years in these herds was playing large role in determining the

performance of lactation traits. The regression line of the phenotypic values of 7345 TD lactation records on herd-year-test-day showed a decrease in the phenotypic trend line as year of TD advanced. Sometimes, ineffective management decisions regarding the culling schemes in the herds were not implemented in the recommended breeding strategy for the studied herds. Moreover, high milk yielding animals had to be disposed during some outbreaks of highly contagious diseases, like brucellosis and tuberculosis … etc. However, the ranges in the values of phenotypic trend of lactation traits decreased unfavorably from 7.49 kg to be 5.69 kg for TDMY, 510 g to be 360 g for TDFY and 284 g to be 223 g for TDPY, associated with unfavorable increase in the phenotypic trends of TDSCS from 1.62 $\log^{10}$ to be 2.43 $\log^{10}$ (**Figure 2**). The decrease in phenotypic trends of all lactation traits over time was suggested to be attributed to low nutritional and feeding levels used and the management practices applied in different herds (**Amin *et al*, 2015, 2021**). Therefore, it is necessary to improve the husbandry/management schemes in herds of the present study. As shown in **Figures 1** and **2**, the genetic and phenotypic trends for lactation traits were irregular, as stated previously in Egyptian buffalo (**El-Bramony, 2014**; **Amin *et al*., 2015, 2021**). In non-Egyptian buffalo studies, the genetic and phenotypic trends obtained for milk yield and components revealed not only decreasing trends (**Chakraborty and Dhaka, 2012; Pawar *et al*., 2018**), but also, other studies reported increasing trends (**Seno *et al*., 2010; Aspilcueta-Borquis *et al*., 2015**; **Nazari *et al*., 2021**).


Genetic Trend for Test-Day Milk Yield (1192 animals), TD= Test-Day
y = -0.005x + 5.1112
R² = 0.0047
Breeding values for TDMY (kg)
Ŷ for breeding values of TDMY (kg)
12
10
8
6
4
2
0
-2
2
1
0
-1
-2
-3
-4
-5
0
NG2003-TD4
NG2003-TD9
NG2006-TD7
NG2010-TD9
NG2021-TD9
NK2003-TD1
NK2006-TD1
NK2010-TD2
NK2011-TD6
NK2021-TD6
NK2022-TD6
EG2005-TD9
EG2008-TD3
EG2009-TD5
Herd-Year-Test-Day
Breeding Values
Genetic Trend
Linear (Breeding Values)
Genetic Trend Line


Genetic Trend for Test-Day Fat Yield (1192 animals), TD= Test-Day
y = -0.0006x + 0.0654
R² = 0.0001
Breeding values for TDFY (kg)
Ŷ for breeding values of TDFY (kg)
0.8
0.6
0.4
0.2
0
-0.2
0.495
0.395
0.295
0.195
0.095
-0.005
0
NG2003-TD4
NG2003-TD9
NG2006-TD7
NG2010-TD9
NG2021-TD9
NK2003-TD1
NK2006-TD1
NK2010-TD2
NK2011-TD6
NK2021-TD6
NK2022-TD6
EG2005-TD9
EG2008-TD3
EG2009-TD5
Herd-Year-Test-Day
Breeding Values
Genetic Trend
Linear (Breeding Values)
Genetic Trend Line


Genetic Trend for Test-day Protein Yield (1192 animals), TD= Test-Day
y = -0.0003x + 0.0602
R² = 0.0001
Breeding values for TDPY (kg)
Ŷ for breeding values of TDPY (kg)
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0.29
0.21
0.13
0.05
-0.03
0
NG2003-TD4
NG2003-TD9
NG2006-TD7
NG2010-TD9
NG2021-TD9
NK2003-TD1
NK2006-TD1
NK2010-TD2
NK2011-TD6
NK2021-TD6
NK2022-TD6
EG2005-TD9
EG2008-TD3
EG2009-TD5
Herd-Year-Test-Day
Breeding Values
Genetic Trend
Linear (Breeding Values)
Genetic Trend Line

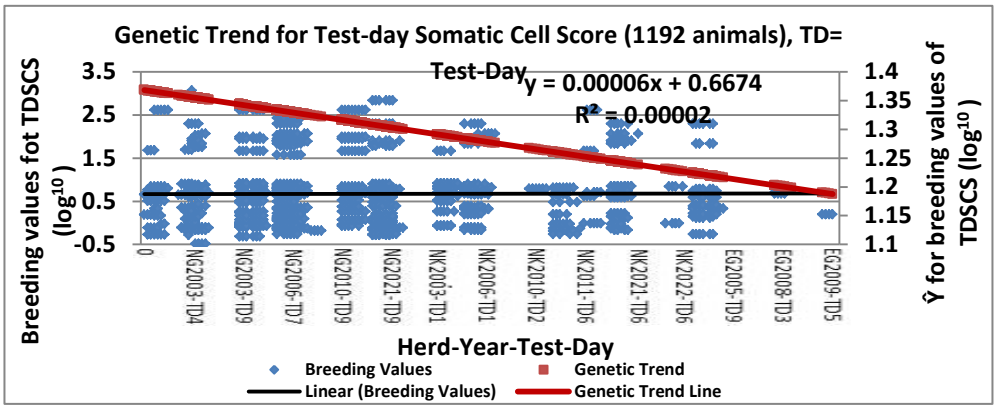
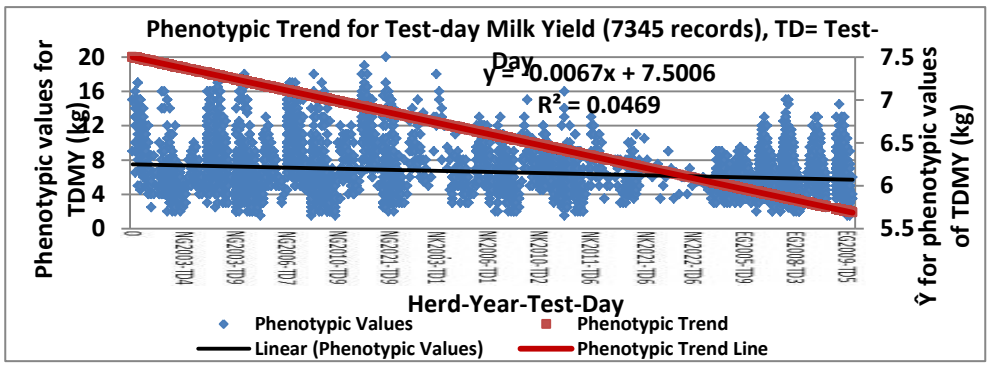
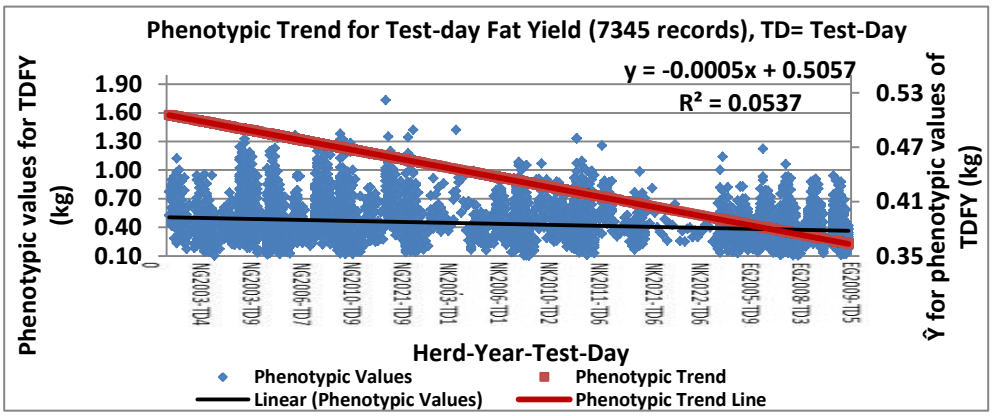
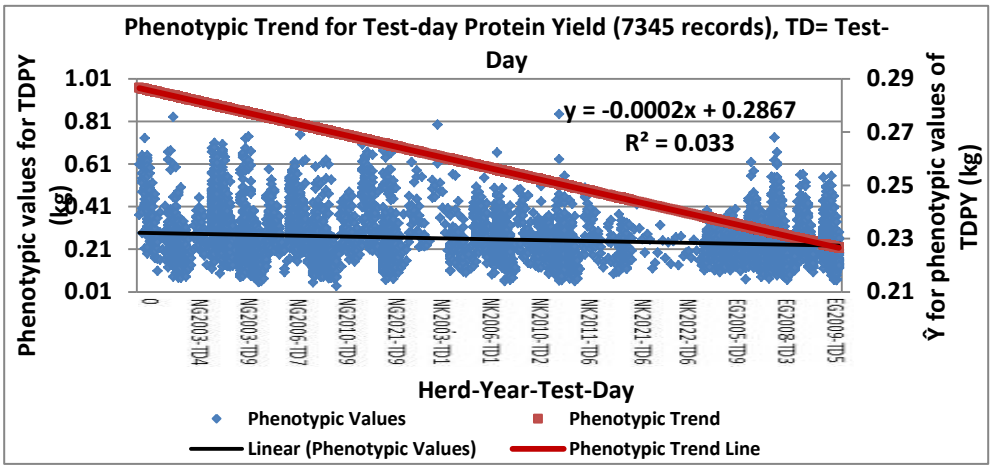


Figure 1. Genetic trends for test-day milk yield (TDMY), fat yield (TDFY), protein yield (TDPY) and somatic cell score (TDSCS) plotted by regressing the breeding values estimated by BLUPF90 software for TD lactation traits on herd-year-test-day of lactation in Egyptian buffalo







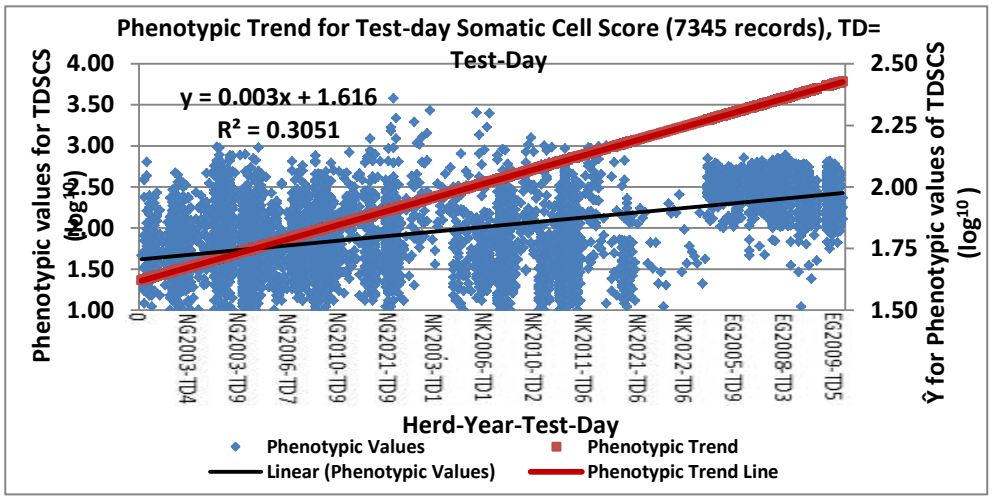


Figure 2. Phenotypic trends for test-day milk yield (TDMY), fat yield (TDFY), protein yield (TDPY) and somatic cell score (TDSCS) plotted by regressing the phenotypic values of TD lactation traits on herd-year-test-day of lactation in Egyptian buffalo

4.2 Reproduction traits in Egyptian buffalo

4.2.1 Descriptive statistics for reproduction traits

The GLSM for reproduction traits were 36.55 *mo*, 99.4 *d* and 385.6 *d* for AFC, DO and CI, respectively (**Table 28**). In an Egyptian buffalo study, the means were 484 *d* for CI and 184 *d* for DO (**Mostafa *et al.*, 2017**). Wide

ranges between minimum and maximum values for reproduction traits in Egyptian buffaloes were observed, being 24.8 to 49.7 *mo* in AFC, 39 to 300 *d* in DO and 300 to 600 *d* in CI (**Table 28**). Furthermore, the coefficients of variation for reproduction traits were mostly moderate or high, 15% for AFC, 76% for DO and 22% for CI. Other studies on Egyptian buffalo indicated that the coefficients of variation for reproduction traits were mostly moderate (), being 27% for CI and 68.1% for DO by **Aziz *et al.*, (2001),** to be 70.46% for DO by **Mostafa *et al*. (2017)**, to be 15.13%, 19.67% and 57.67% for AFC, CI and DO, respectively by **Helmy and Somida (2021)**.

Table 28. The generalized least square means (GLSM), standard deviations (SD), standard errors (SE), minimum and maximum values and coefficients of variation (CV) for reproduction traits in Egyptian buffalo

Reproduction trait	GLSM	SD	SE	Minimum value	Maximum value	CV
AFC, month **(N= 1951 records)**	36.55	5.31	0.120	24.8	49.7	15
DO, day **(N= 7279 records)**	99.4	75.30	0.883	39	300	76
CI, day **(N= 7279 records)**	385.6	83.90	0.983	300	600	22

AFC= Age at first calving; DO= Days open; CI= Calving interval.

4.2.2 Heritability estimates and permanent environmental effects for reproduction traits

The heritability estimated by single-trait animal model for reproduction traits were low, being 0.10 for AFC, 0.02 for DO and 0.02 for CI (**Table 29**). The proportions of permanent environmental effects (p^2) were also low for DO and CI, being 0.02 and 0.01, respectively (**Table 29**). In several Egyptian buffalo studies, the heritability estimates of reproduction traits were mostly low or rarely moderate, being 0.12 to 0.35 for AFC, 0.002 to 0.19 for CI and 0.0001 to 0.18 for DO (**El-Bramony, 2014; Mostafa *et***

***al*., 2017; Shafik *et al*., 2017; El-Bramony *et at*., 2017; Amin *et al*., 2021; Helmy and Somida, 2021; Easa *et al*., 2022**).

Table 29. Heritabilities (h^2) and proportions of permanent environmental effects (p^2) and random error effects (e^2) for reproduction performance in Egyptian buffalo

Reproduction traits	h^2±SE	p^2±SE	e^2±SE
AFC, month **(N= 1951 record)**	0.10 ±0.043	---	0.92 ±0.04
DO, day **(N= 7279 record)**	0.02 ±0.01	0.02 ±0.01	0.96 ±0.01
CI, day **(N= 7279 record)**	0.02 ±0.01	0.01 ±0.01	0.97 ±0.01

AFC= Age at first calving; DO= Days open; CI= Calving interval; SE= standerd error.

4.2.3 Predicted breeding values (PBV) for reproduction traits

Estimates of minimum and maximum PBVs and their accuracy of predictions (r_A) and ranges for AFC, DO and CI are given in **Table (30)**. The ranges in PBVs were moderate or high -8.24 to 10.84 *mo* for AFC, -124.7 to 123.9 *d* for DO and -141.8 to 132.5 *d* for CI. The accuracies (r_A) of minimum and maximum estimates of PBVs were moderate or high in most reproduction traits, ranging from 0.791 to 0.999 (**Table 30**). However, the negative PBVs for reproduction traits are desired for selection purposes. The percentages of the experimental animals having negative PBVs for AFC, DO and CI were 47, 37 and 36 %, respectively (positive PBVs % are presented in **Table 30**). The ranges in PBVs available in literature for reproduction traits in Egyptian buffalo were high, ranging from -15.8 to 143 *d* for AFC and -43.1 to 97.9 *d* for DO (**Shalaby *et al*., 2016; Shafik *et al*., 2017; Abo-Gamil *et al*., 2017; Amin *et al*., 2021**). Therefore, using the breeding values for AFC and lactation traits (milk, fat, protein and somatic cell score) in selection program will reduce the generation interval and increase the productive period in the Egyptian buffalo, while using the breeding values for CI or DO in selection could attain limited improvement in these reproduction traits.

Table 30. Minimum and maximum predicted breeding values (PBVs), standard errors (SE) and accuracy of predictions (r_A) for reproduction traits in Egyptian buffalo estimated by single-trait Animal Model using BLUPF90 software

Traits	Minimum PBV			Maximum PBV			Range in PBV	Positive PBV (%)
	PBV	SE	r_A	PBV	SE	r_A		
Reproduction traits (N= 3285 animals with 7279 records)								
AFC (month)	-8.24	0.393	0.950	10.84	0.321	0.791	19.08	53
DO (day)	-124.7	0.735	0.996	123.9	0.409	0.998	248.7	63
CI (day)	-141.8	0.735	0.997	132.5	0.409	0.999	274.3	64

SE= standerd error; AFC= Age at first calving; DO= Days open; CI= Calving interval.

4.2.4 Genetic and phenotypic trends for reproduction traits:

Across the years from 2002 to 2023 in the experimental herds of the present study, the genetic trends plotted for reproduction traits are shown in **Figure 3**. The regression line of breeding values for reproduction traits of 3285 animals (buffaloes with record and sires and dams without records) showed favorable decrease in the genetic trend line over time of calving. Also, the ranges of the genetic trends for AFC, DO and CI were favorably decreased from 0.24 *mo* to be -0.14 *mo*, 5.5 *d* to be 2.9 *d* and 6.9 *d* to be 3.6 *d*, respectively. The positive genetic trends plotted for all reproduction traits resulting from selection applied in these experimental herds. The present results and previous Egyptian reports (**El-Bramony, 2014; Amin *et al*., 2015** and **2021**) gave evidence that genetic improvement in buffalo herds is limited despite of the frequent attempts made to improve reproduction traits. This is due to the following reasons: 1) insufficient or lack of recording induced difficulty to keep track of genealogical aspects, 2) natural insemination was applied and practiced in APRI research herds and consequently the planned progeny test could not be performed accurately, and 3) the technology of artificial insemination is not widespread at the field levels. The above-mentioned reasons are of considerable contribution to slow-down the Egyptian buffalo genetic improvement for reproduction traits. In fact, buffalo estrus is not detectable easily and inseminations were often offered at the wrong time, causing low pregnancy rates and seasonal anestrus and therefore the buffalo producers are afraid of missing detection of heat period.

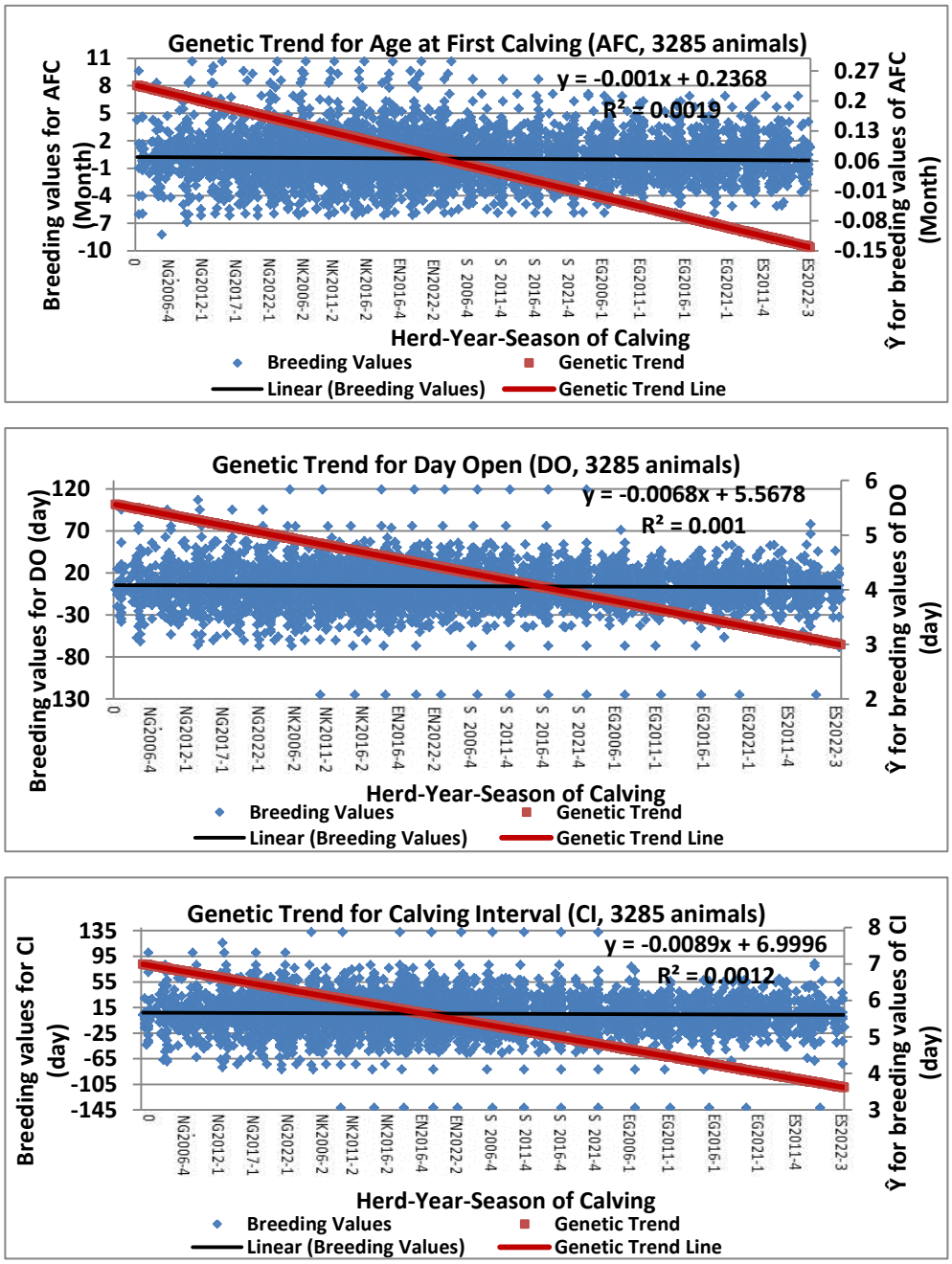


Figure 3. Genetic trends for reproduction traits plotted by regressing the breeding values estimated by BLUPF90 software for age at first calving (AFC), day open (DO) and calving interval (CI) on herd-year season of calving in Egyptian buffalo

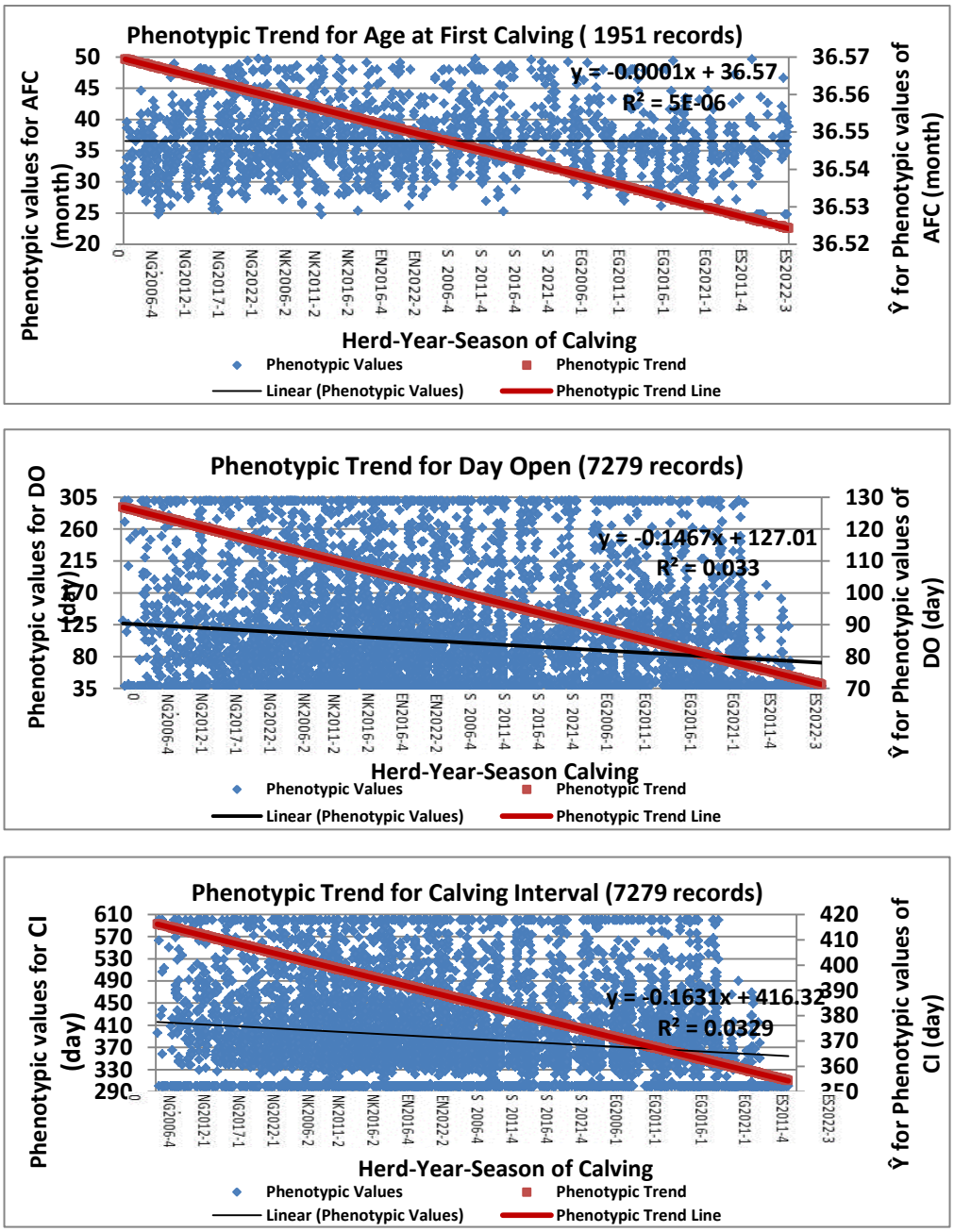


Figure 4. Phenotypic trends for reproduction traits plotted by regressing the phenotypic values for age at first calving (AFC), day open (DO) and calving interval (CI) on herd-year season of calving in Egyptian buffalo

The regression line of phenotypic values on 1951 records for AFC or on 7279 records for DO and CI revealed favorable decreasing in phenotypic trend over time (**Figure 4**). Wide ranges in the phenotypic values of reproduction traits in herd-year-season of calving subclasses were observed, being 36.57 *mo* to be 36.52 *mo* for AFC, 127 *d* to be 71 *d* for DO and 416 *d* to be 354 *d* for CI. The genetic and phenotypic trends for AFC and CI were increasing together as cited by **Kour and Narang (2021)** in Murrah buffalo, while reversible trends were observed by **Amin *et al.* (2021)** in Egyptian buffalo where the genetic trend was increasing, and the phenotypic trend was decreasing or *vice versa*. **Bashir *et al.* (2009)** in Nili-Ravi buffalo in Pakistan showed favorable decreasing in genetic trend for AFC, while **Gupta *et al.* (2015)** reported unfavorable increase in genetic trend for AFC in Indian Murrah buffalo. In Egyptian buffalo, **Shalaby *et al.* (2016)** reported that the genetic and phenotypic trends for DO and CI decreased favorably, while the results of **Amin *et al.* (2021)** indicated unfavorable increase in the genetic trends for these traits.

4.3 Semen traits in Egyptian buffalo

4.3.1 Descriptive statistics, heritabilities and permanent environmental effects for semen traits

The generalized least square means (GLSM), standard error (SE), standard deviations (SD), minimum and maximum values, coefficients of variation (CV %), heritabilities and permanent environmental effects for semen traits are shown in **Table (31)**. The GLSM for EV, MS, LS, AS and SC were 3. 7 *ml*, 63.8 %, 62.9 %, 5.06 % and 0.83×10^9 sperms per *ml*, respectively. These GLSM were in accordance with those means previously reported by several Egyptian investigators for Egyptian buffalo **(Khattab *et al.*, 2015; Kadoom *et al.*, 2016; Rushdi *et al.*, 2017; Amin *et al.*, 2024).** In this regard, **Kadoom *et al.* (2016**) reported 60.5 % for LS and 16.7 % for AS, while **Rushdi *et al.* (2017)** specified 66.20 % for MS trait and 15.15 % for AS trait. The ranges between minimum and maximum values for semen traits in Egyptian buffalo were high, being 1.0 to 10.5 *ml* for EV, 10 to 95 % for MS, 10 to 88 % for LS, 3 to 44 % for AS and 0.2 to 3.8×10^9 sperms per *ml*

for SC (**Table 31**). The coefficients of variation (CV%) for semen traits were moderate or high, being 46% for EV, 28% for MS, 27% for LS, 55% for AS and 50% for SC (**Table 31**). Similarly, wide variation for semen traits in Egyptian buffalo were reported by **Khattab *et al.* (2015)**, being 38.61% for AS, 21.86 % for LS and 26 % for MS. Also, **Salem *et al.* (2023)** and **Amin *et al.* (2024)** reported CVs of 46.57% for EV, 25.17% for MS, 25.17% for LS, 43.53% for AS and 24.49% for SC in the Egyptian buffalo. Moreover, **El Basuini *et al*. (2024)** evaluating some semen traits in Egyptian buffalo, stated that CV% were 38.7, 21.83 and 25.93% for EV, LS and total motility, respectively.

The heritability estimates for semen traits were moderate, being 0.17, 0.28, 0.27, 0.27 and 0.23 for EV, MS, LS, AS and SC, respectively (**Table 31**), *i.e.* selection for semen traits in Egyptian buffalo could be performed efficiently. In Egyptian buffalo, the heritability estimates for semen traits were mostly moderate and ranged from 0.08 to 0.40 for EV, 0.06 to 0.42 for MS, 0.09 to 0.41 for LS, 0.04 for AS and 0.46 to 0.49 for SC (**El-Basuini, 2010; Khattab *et al.*, 2015; Salem *et al.*, 2023)**. In accordance with the present results, **El Basuini *et al*. (2024)** reported heritability estimates of 0.08, 0.27 and 0.24 for EV, LS and total motility traits in Egyptian buffalo. However, these estimates varied from one study to another and these differences in heritability estimates for semen traits may be attributed to several factors such as the fixed effects and covariates considered in the model of analysis, structure of data used, genetic constitution of the buffalo type, and coefficients of inbreeding and the relationship coefficient matrix.

Table 31. Descriptive statistics, heritabilities (h^2), proportions of permanent environmental effects (p^2) and random error effects (e^2) for semen traits of Egyptian buffalo

Item	EV (*ml*)	MS (%)	LS (%)	AS (%)	SC (10^9 sperms per ml)
Descriptive statistics[+]:					
GLSM	3.7	63.8	62.9	5.06	0.83
SD	1.72	17.6	16.9	2.77	0.41
SE	0.02	0.25	0.23	0.04	0.002
Minimum	1	10	10	3	0.2
Maximum	10.5	95	88	44	3.8
Coefficient of variation (CV)	46	28	27	55	50
Heritability estimates and permanent environmental and random error effects:					
h^2±SE	0.17±0.05	0.28±0.08	0.27±0.07	0.27±0.09	0.23±0.07
p^2±SE	0.16±0.03	0.37±0.06	0.35±0.05	0.43±0.07	0466±0.07
e^2±SE	0.67±0.03	0.35±0.04	0.38±0.04	0.30±0.04	0.31±0.02

Total number of records= 5178; EV= ejaculate volume, MS= motility of sperms, LS= live sperms, AS= abnormal sperms and SC= sperms cell concentration.

[+]GLSM= Generalized least square means (GLSM) estimated by Animal Model using PEST software, SD= standard deviations, SE=Standard error.

The proportions of permanent environmental effects (p^2) were moderate for EV, MS, LS, AS and SC, being 0.16, 0.37, 0.35, 0.43 and 0.46, respectively (**Table 31**) **Salem *et al*. (2023)** showed that the proportion of permanent environmental effects for EV, MS, LS, AS and SC in Egyptian buffalo were low or moderate, being 0.06, 0.30, 0.29, 0.024 and 0.0.29, respectively. In Holstein dairy bulls, **Mathevon *et al*. (1998)** reported that the permanent environmental effects were mostly moderate and ranged from 0.0 to 0.22 in bulls younger than 30 mo and ranged from 0.0 to 0.63 in mature bulls aged from 4 to 6 years old for ejaculate volume, sperms concentration, motility of spams and total sperms. .

.4.3.2 Predicted breeding value (PBV) for semen traits

Estimates of minimum and maximum PBVs and their accuracy of predictions (r_A) for semen traits are given in **Table (32)**. The ranges in PBVs were moderate or high, being -0.63 to 0.42 *ml* for EV, -27.3 to 85.0 % for MS, -27.3 to 81.7 % for LS, -3.7 to 24.8 % for AS and -1.23 to 2.5×10^9 sperms per ml for SC. The percentages of positive PBVs for bulls with records and sires and dams of bulls without records for semen traits were high, ranging from 83 to 87 % (**Table 32**). The accuracies (r_A) of minimum and maximum PBVs for semen traits were high, ranging from 0.62 to 0.96 **(Table 32)**. Thus, high genetic variabilities in semen traits suggested that there are promising prospects for selecting Egyptian buffalo bulls to enhance semen traits. Similarly, the reviewed ranges in PBVs were -0.448 to 3.32 *ml* for EV, -4. 28 to 52 % for MS, -5.85 to 8.10 % for LS and 799 to 1959 million per ml for SC in Egyptian buffalo (**El-Basuini, 2010)** and in Indian Murrah buffalo (**Kumar *et al.*, 2023)**. In cattle studies, the ranges in breeding values were -7.10 to 11.0 *ml* for EV, -16.97 to 11.62 % for MS and -336 to 428×10^6 sperm per *ml* for SC **(Olsen *et al.*, 2020; Butler *et al.*, 2021; Khattab *et al.*, 2022)**.

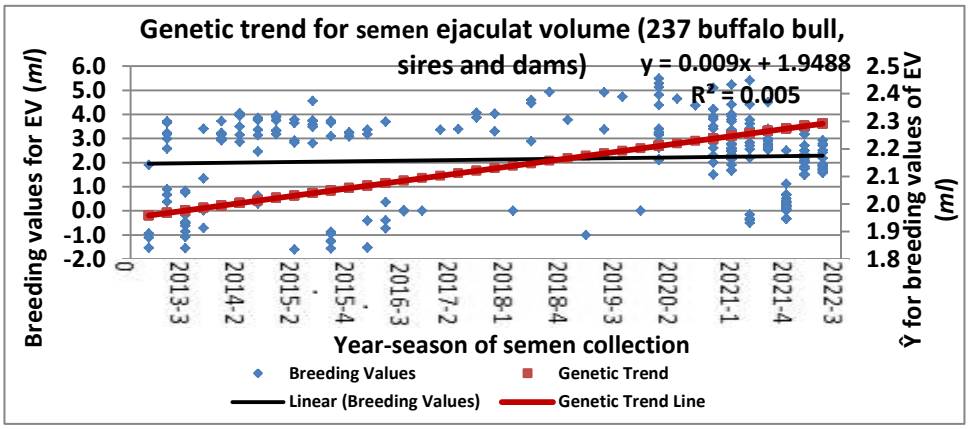
Table 32. Minimum and maximum predicted breeding values (PBV), their standard errors (SE) and accuracy of predictions (r_A) for semen traits in Egyptian buffalo estimated by Single-trait Animal Model using BLUPF90 software

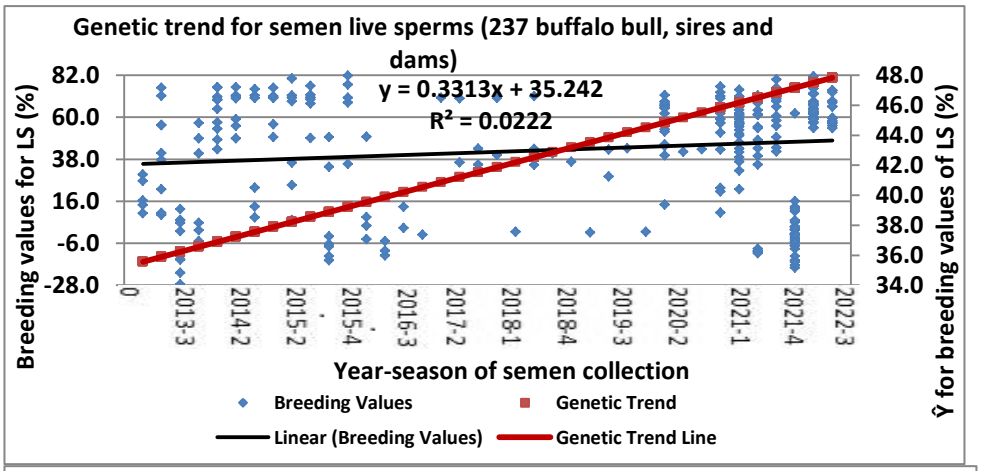
Trait	Minimum PBV	SE	r_A	Maximum PBV	SE	r_A	Range in PBV	Positive PBV (%)
EV (*ml*)	-0.63	0.35	0.62	0.42	0.34	0.64	1.05	87
MS (%)	-27.3	2.6	0.95	85.0	5.3	0.78	112.3	85
LS (%)	-27.2	2.2	0.95	81.7	4.5	0.83	108.9	86
AS (%)	-3.7	2.1	0.60	24.8	1.5	0.81	28.2	83
SC (10^9 sperms per ml)	-1.3	0.07	0.96	2.65	0.15	0.83	3.95	84

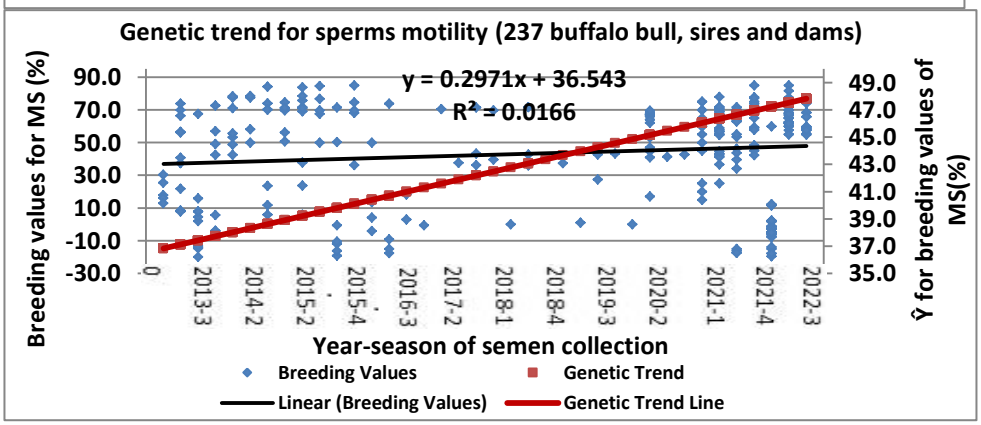
Number of animals used = 237.

4.3.3 Genetic and phenotypic trends for semen traits

The genetic trends plotted for semen traits during the years from 2013 to 2022 are shown in **Figure 5**. The range of genetic trends for semen traits were favorably increased over time from 1.99 to 2.3 *ml* for EV, 36.8 to 47.8 % for MS, 35.6 to 47.8 % for LS, 2.3 to 5.9% for AS and 0.39 to 1.24×10^9 sperms per ml for SC. These wide ranges in genetic trends reflected suitable methodology of culling and replacement presses practiced in buffalo herds of the present study. The positive genetic trends for EV, MS, LS and SC traits were resulting from selection program practiced for semen traits (**Figure 5**). The phenotypic trends plotted for EV, MS and LS traits throughout the experimental period decreased from 4.1 to 3.1 *ml* for EV, 68.2 to 57.1% for MS trait and 67.4 to 56.2 for LS traits and increased from 3.1 to 8.1% for AS traits and 0.6 to 1.3×10^9 sperm per *ml* for SC traits (**Figure 6**). The decrease in phenotypic trend in EV, MS and LS traits may be attributed to low nutritional level applied and management practiced in the two herds of the present study. Studies in buffalo **(Kumar *et al.*, 2023)** and cattle **(Olsen *et al.*, 2020)** have shown that genetic and phenotypic trends for semen traits were favorable and showing considerable increase in both trends. **Olsen *et al.* (2020)** found that the genetic trends in EV, MS and SC traits were increased in Norwegian Red cattle. **Kumar *et al.* (2023)** showed that genetic and phenotypic trends were positive and showing favorable increase in EV and MS traits in Indian Murrah buffalo.


Genetic trend for semen ejaculat volume (237 buffalo bull, sires and dams)
y = 0.009x + 1.9488
R² = 0.005
Breeding values for EV (ml)
Ŷ for breeding values of EV (ml)
Year-season of semen collection
Breeding Values
Genetic Trend
Linear (Breeding Values)
Genetic Trend Line


Genetic trend for semen live sperms (237 buffalo bull, sires and dams)
y = 0.3313x + 35.242
R² = 0.0222
Breeding values for LS (%)
Ŷ for breeding values of LS (%)
Year-season of semen collection
Breeding Values
Genetic Trend
Linear (Breeding Values)
Genetic Trend Line


Genetic trend for sperms motility (237 buffalo bull, sires and dams)
y = 0.2971x + 36.543
R² = 0.0166
Breeding values for MS (%)
Ŷ for breeding values of MS(%)
Year-season of semen collection
Breeding Values
Genetic Trend
Linear (Breeding Values)
Genetic Trend Line

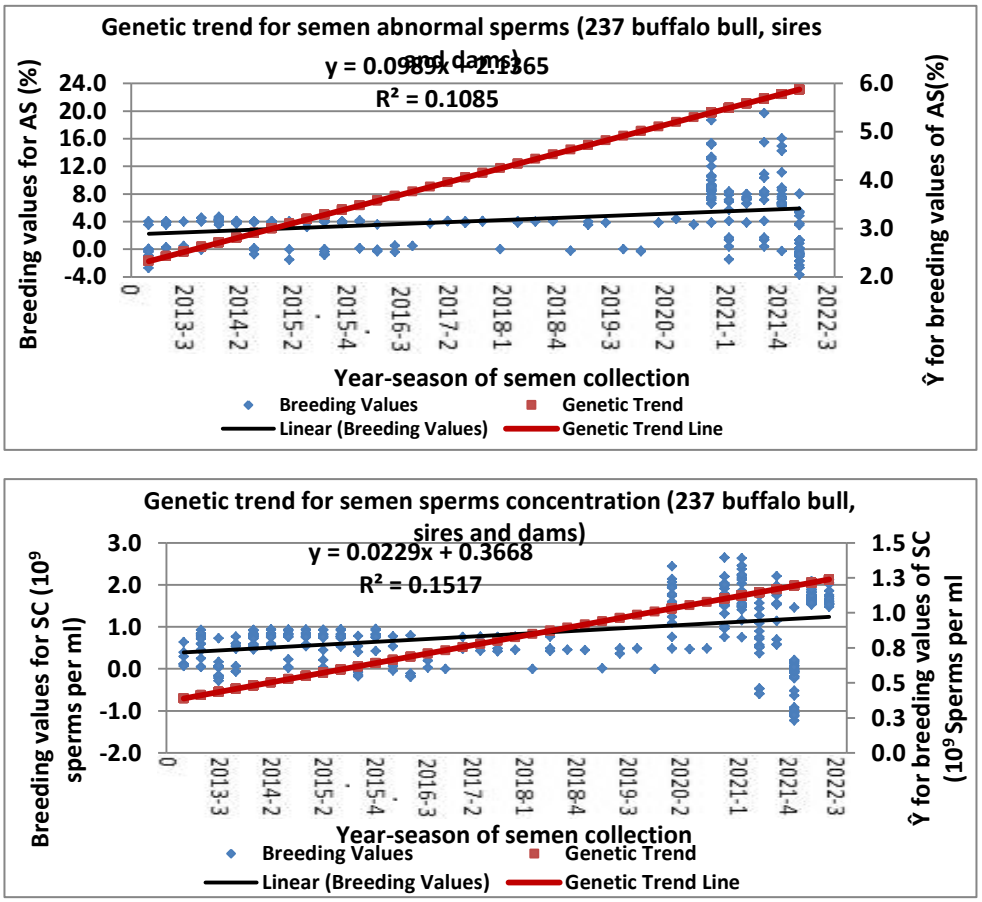
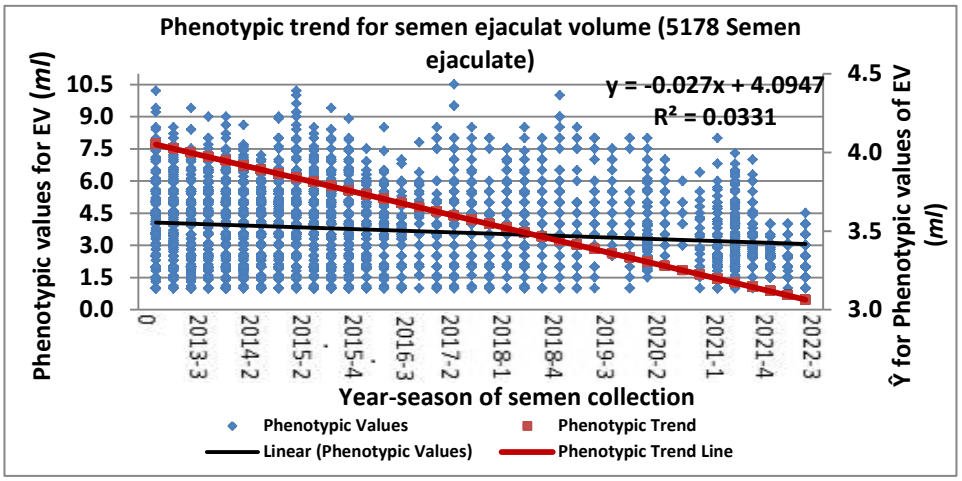
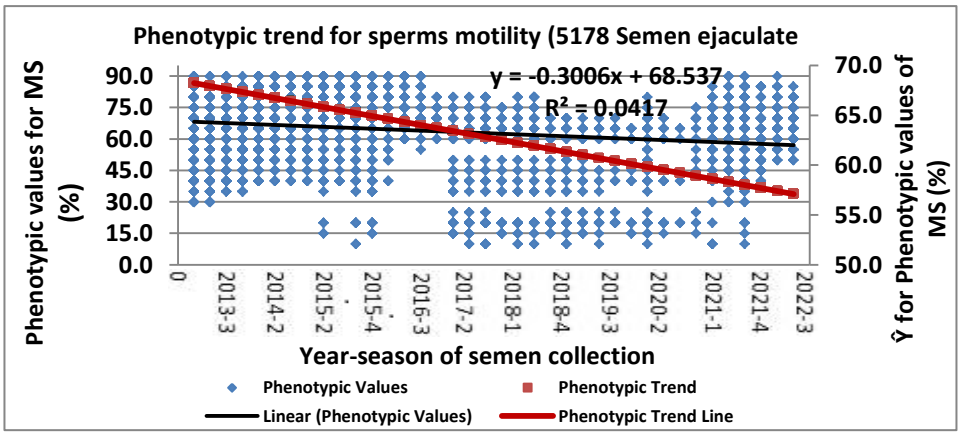
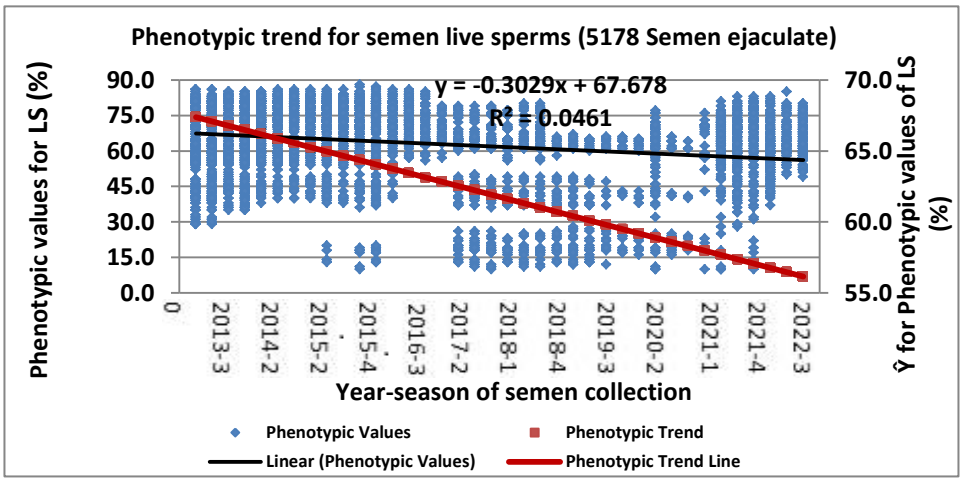


Figure 5. Genetic trends for ejaculate volume (EV), sperms motility (MS), live sperms (LS), abnormal sperms (AS) and sperms concentration (SC) plotted by regressing the breeding values of semen trait on year-season of semen collection estimated by BLUPF90 software in Egyptian buffalo


Phenotypic trend for semen ejaculat volume (5178 Semen ejaculate)
y = -0.027x + 4.0947
R² = 0.0331
Phenotypic values for EV (ml)
Ŷ for Phenotypic values of EV (ml)
Year-season of semen collection
Phenotypic Values
Phenotypic Trend
Linear (Phenotypic Values)
Phenotypic Trend Line


Phenotypic trend for sperms motility (5178 Semen ejaculate
y = -0.3006x + 68.537
R² = 0.0417
Phenotypic values for MS (%)
Ŷ for Phenotypic values of MS (%)
Year-season of semen collection
Phenotypic Values
Phenotypic Trend
Linear (Phenotypic Values)
Phenotypic Trend Line


Phenotypic trend for semen live sperms (5178 Semen ejaculate)
y = -0.3029x + 67.678
R² = 0.0461
Phenotypic values for LS (%)
Ŷ for Phenotypic values of LS (%)
Year-season of semen collection
Phenotypic Values
Phenotypic Trend
Linear (Phenotypic Values)
Phenotypic Trend Line

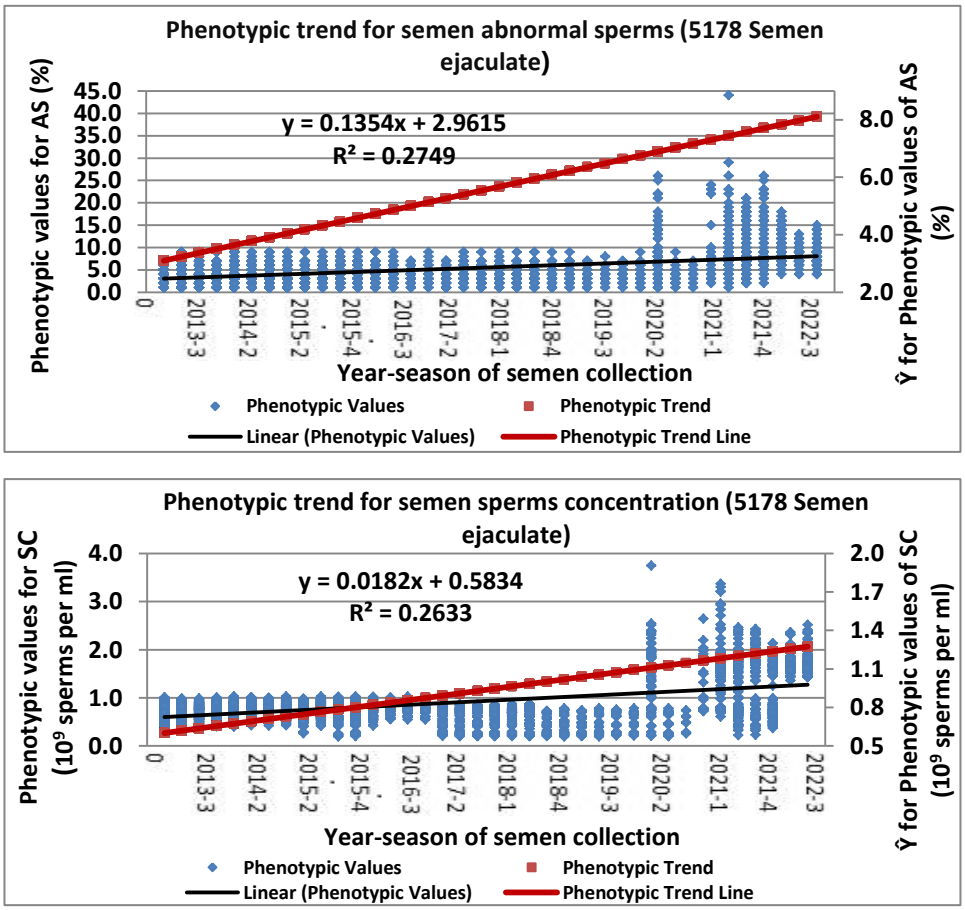


Figure 6. Phenotypic trends for ejaculate volume (EV), sperms motility (MS), live sperms (LS), abnormal sperms (AS) and sperms concentration (SC) plotted by regressing the phenotypic values of semen traits on year-season of semen collection in Egyptian buffalo

4.4 Growth traits in Egyptian buffalo

4.4.1 Descriptive statistics, heritabilities and maternal common environmental effects for growth traits

The GLSM, standard deviations (SD), standard error (SE), minimum and maximum values, coefficients of variation (CV %), heritability estimates

and proportion of the common environmental effects for BW, WW and DG are shown in **Table (33)**. The GLSM for BW, WW and DG were 35.0 *kg*, 94.7 *kg* and 0.616 *kg*, respectively. In other studies, on Egyptian buffalo, lower means of 33 *kg* for BW was reported by **El-Awady *et al.* (2005)**, 87 *kg* for WW was reported by **Ashmawy and El-Bramony (201**7) and 32.78 *kg* for BW and 91.96 *kg* for WW by **El-Den *et al*. (2020)**. The ranges between minimum and maximum values of body weights and gains in Egyptian buffalo in the present study were high, being 15 to 53 *kg* for BW, 50 to 147 *kg* for WW and 0.10 to 1.40 *kg* for DG, with coefficients of variation of 18, 13 and 24 % for BW, WW and DG, respectively **(Table 33)**. In this respect, moderate or high coefficient of variation was reported by **Easa *et al*. (2022)** for BW in Egyptian buffalo (15.5%), while it was 23.0 % for WW in Colombian buffalo (**Agudelo-Gómez *et al*., 2015**).

The heritability values estimated by animal model for BW, WW and DG were mostly moderate or high, being 0.26, 0.50 and 0.55, respectively (**Table 33**), indicating that the Egyptian buffalo herds in the present study were not subjected to intensive programmes of selection. Therefore, there is a future possibility for successful direct selection on body weights and gains in the studied buffalo populations. However, the heritability estimates for BW and WW were mostly similar to those estimates cited in Egyptian buffalo studies (**EL-Awady *et al*., 2005; Shahin *et al*., 2010; Ashmawy and El-Bramony, 2017; Elsayed *et al*., 2021; El-Den *et al*., 2020; Salem *et al*., 2020; Easa *et al*., 2022**) and in Murrah and Nili-Ravi buffalo studies in Brazil, India, Pakistan, Colombia and Italy (**Cassiano *et al*., 2004; Suhail *et al*., 2009; Malhado *et al*., 2012; Gupta *et al*., 2015; Agudelo-Gómez *et al*., 2015; Rezende *et al*., 2020**). Differences among estimated and reviewed heritabilities may be attributed to the structure and genetic variation of the studied populations, method of variance components estimation and model of analysis (**Malhado *et al*, 2012**) and environmental deviations, large standard errors due to small datasets as well as to the fact that body weights and gains are strongly influenced by the management scheme and due to possible variation in seasonal supply of green feedstuffs.

Table 33. Descriptive statistics, heritabilities (h^2), proportions of maternal common environmental effects (c^2) and random error effects (e^2) for growth traits of Egyptian buffalo

Item	BW (*kg*)	WW (*kg*)	DG (*kg*)
Descriptive statistics[+]:			
Numbers of valves	8229	8203	8181
GLSM	35.0	94.7	0.616
SD	6.32	12.3	0.151
SE	0.69	0.14	0.002
Minimum value	15	50	0.10
Maximum value	53	147	1.40
Coefficient of variation (CV %)	18	13	24
Heritability estimates and maternal common environmental effects estimated by Single-trait Animal Model:			
h^2±SE	0.26±0.036	0.50±0.016	0.55±0.019
c^2±SE	0.23±0.008	0.34±0.015	0.24±0.014
e^2±SE	0.51±0.035	0.12±0.013	0.19±0.018

BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.

[+]GLSM= Generalized least square means (GLSM) estimated by Animal Model using PEST software, SD= standard deviations, SE=Standard error.

The proportions of maternal common environmental effects (c^2) were moderate for BW, WW and DG, being 0.23, 0.34 and 0.24, respectively (**Table 33**). The variation in WW due to maternal common environmental effects was also moderate but higher than the value for BW, indicating that the common environmental influence of the buffalo dam has considerable maternal carry over environmental effects on calves from birth to weaning, *i.e.* the maternal common environmental effects of buffalo dams were dominant from birth until weaning. In this regard, **Cassiano *et al.* (2004)** reported that the maternal common environment effects for birth weight were low or medium being 0.11, 0.17, 0.37 and 0.04 for Carabao, Jaffarabadi, Mediterranean and Murrah buffalo, respectively. **Malhado *et al.* (2007)** showed that maternal common environmental effects on body weight at 205 days were high (0.43) in Brazilian buffalo.

4.4.2 Predicted breeding values (PBV) for growth traits

The estimates of minimum and maximum PBV and their accuracies of predictions (r_A) for BW, WW and DG are given in **Table (34)**. Wide variations in PBVs of 10681 animals were observed, ranging from -4.2 to 3.5 *kg* for BW, -42.4 to 44.2 *kg* for WW and -0.44 to 0.52 *kg* for DG. The percentages of animals with positive PBVs (buffalo calves with records and sires and dams without records) for body weights and gains were high ranging from 54 to 59 % (**Table 34**). Thus, the high genetic variabilities in body weights and gains indicated that there are good opportunities to improve these traits in Egyptian buffalo through selection. Similar wide variations in PBVs were observed in some buffalo studies (**EL-Awady *et al.*, 2005** and **Agudelo-Gómez *et al.*, 2015**). In the Egyptian buffaloes, **EL-Awady *et al.* (2005)** found that the ranges in PBVs for calves were high ranging from -4.8 to 3.4 *kg* for BW, -15.8 to 9.7 *kg* for WW and -131 to 99 *g* for DG, associated with high ranges in PBVs for sires (-2.3 to 2.6 *kg* for BW, -6.4 to 15.5 *kg* for WW and -79.9 to 116 *g* for DG) and also high ranges in PBVs for buffalo dams (-2.9 to 2.1 *kg* for BW, -10.6 to 15.5 *kg* for WW and -111 to 118 *g* for DG). On the contrary, **Elsayed *et al.* (2021)** and **Salem *et al.* (2020)** reported that the ranges in PBVs were low and ranged from -0.02 to 0.2 *kg* for BW and -0.02 to 0.5 *kg* for WW.

The accuracies (r_A) of minimum and maximum PBVs for body weights or gains were high, ranging from 0.63 to 0.89 (**Table 34**). These high accuracies may be due to that heritabilities for body weights and gains were highly associated with more available pedigree information for the studied buffalo calves along with their sires and dams (**EL-Awady *et al.*, 2005; Elsayed *et al.*, 2021; Salem *et al.*, 2020**). However, high accuracies in PBVs obtained in the present study indicated that selection of the buffalo calves in these herds could be used as parents in the next generations, and this would lead to sustainable genetic improvement for growth traits in Egyptian buffalo.

Table 34. Minimum and maximum predicted breeding values (PBV), their standard errors (SE) and accuracies of predictions (r_A) for growth traits in Egyptian buffalo estimated by Single-trait Animal Model using BLUPF90 software

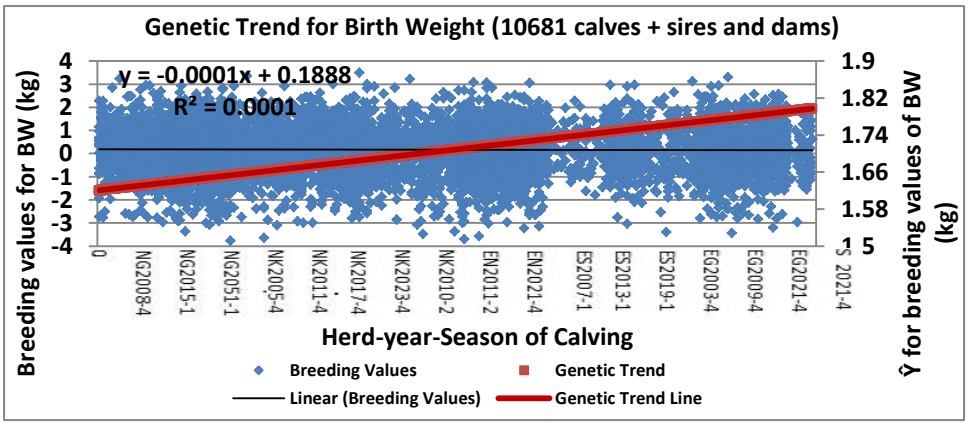
Trait	Minimum PBV	SE	r_A	Maximum PBV	SE	r_A	Range in PBV	Positive PBV (%)
BW (*kg*)	-4.2	1.69	0.65	3.5	1.73	0.63	7.70	59
WW (*kg*)	-42.4	4.47	0.89	44.2	4.86	0.88	86.6	54
DG (*kg*)	-0.44	0.065	0.84	0.52	0.07	0.83	0.96	56

No. of animals = 10681; SE=Standard error; BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.

4.4.3 Genetic and phenotypic trends for growth traits

The genetic trends plotted for BW, WW and DG across the years from 2003 to 2024 are shown in **Figure 7**. The regression line of PBVs for body weights and gains of 10681 animals are showing slight increase in the genetic trends as year-season of calving advanced, the ranges increased slightly from 1.6 to 1.8 *kg* for BW, -0.519 to 1.57 *kg* for WW and -24 to 18 *g* for DG. **Gupta *et al.* (2015)** showed that genetic trend for WW in Murrah buffalo increased as year of calving advanced. But, the wide ranges in the genetic trends (**Figure 7**) reflected precise methodology of culling and replacement processes performed in the studied herds. However, the slight increase in genetic trends registered in the present study over 22 years period can be justified by the facts that: 1) Progeny testing of selection was not practiced properly or not performed on a large scale, 2) Selection towards the desired changes over 22 years was not effective enough due to the lack of efficient selection or breeding methods to evaluate the calves, 3) Herds size were small, 4) Inbreeding was practiced in few cases, 5) lack of accuracy in performance recording, 6) Few elite sires were used in the breeding strategy in the recent years, 7) Small set of random mating was practiced in some small herds, and 8) Young calves were selected on the basis of growth rate without considering their breeding values.

The phenotypic trends plotted for body weights and gains during the period from 2003 to 2024 are shown in **Figure 8**. The ranges in phenotypic values for BW (8229 calves), WW (8203 calves) and DG (8181 calves) showed non-favorable decreasing in values of the phenotypic trend as year-season of calving advanced. The ranges in phenotypic values of herd-year-season of calving for body weights and gains decreased slightly from 36.6 to 32.9 *kg* for BW, 94.55 to 94.15 *kg* for WW and 628 to 582 *g* for DG. This slight decrease in phenotypic trends of all weights and gains may be attributed to low nutritional and feeding levels applied and unsuitable management schemes practiced in different herds. In the buffalo literature, the genetic and phenotypic trends for BW and WW were favorable showing an increase in body weights and gains as stated in Brazilian buffalo (**Malhado *et al.*, 2007**), in Murrah buffalo (**Gupta *et al.*, 2015**) and in Egyptian buffalo **(El-Bramony, 2014; Elsayed *et al.*, 2021; Salem *et al.*, 2021**).



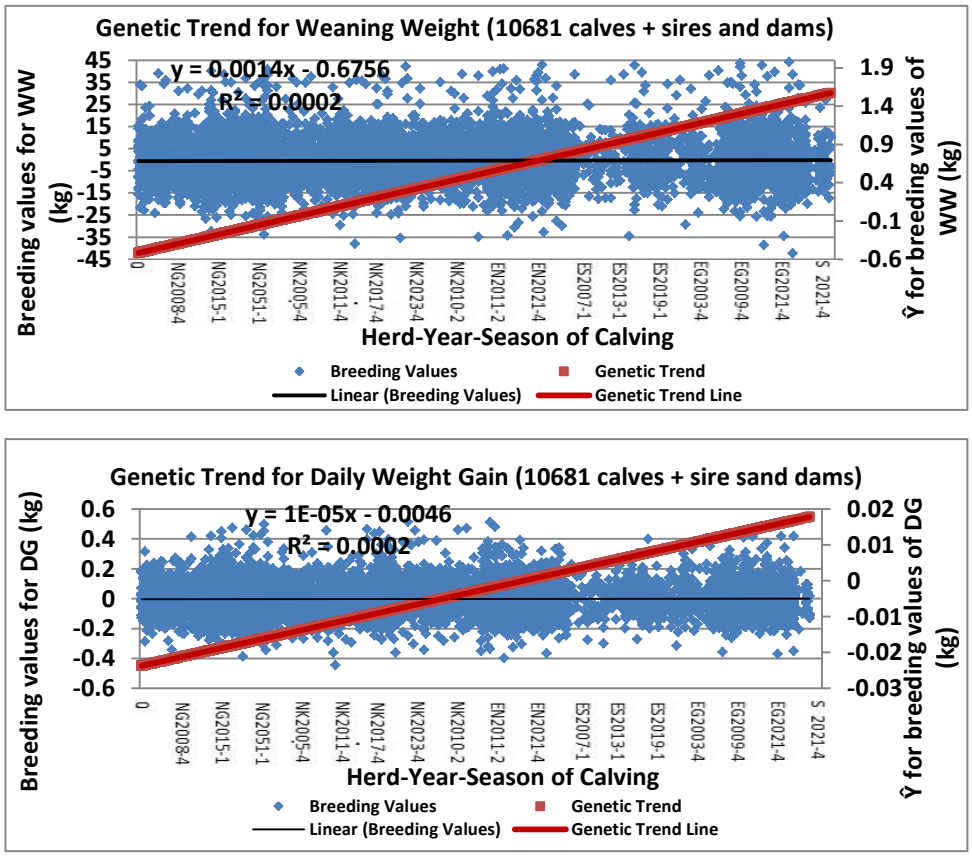
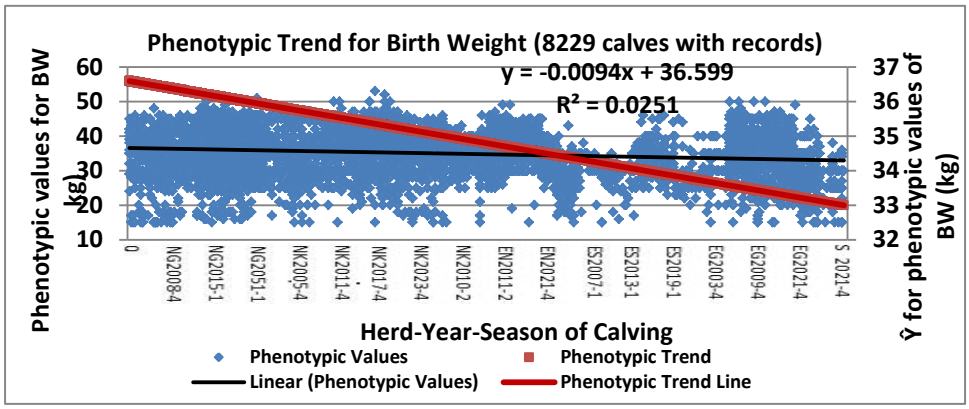
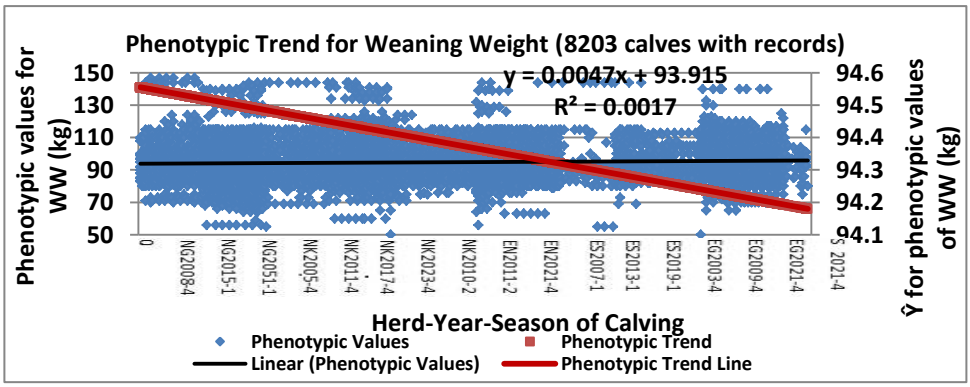


Figure 7. Genetic trends for birth weight (BW), weaning weight (WW) and daily weight gain (DG) in Egyptian buffalo plotted by regressing the breeding values estimated by BLUPF90 software of growth trait on herd-year season of calving in El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S) herds





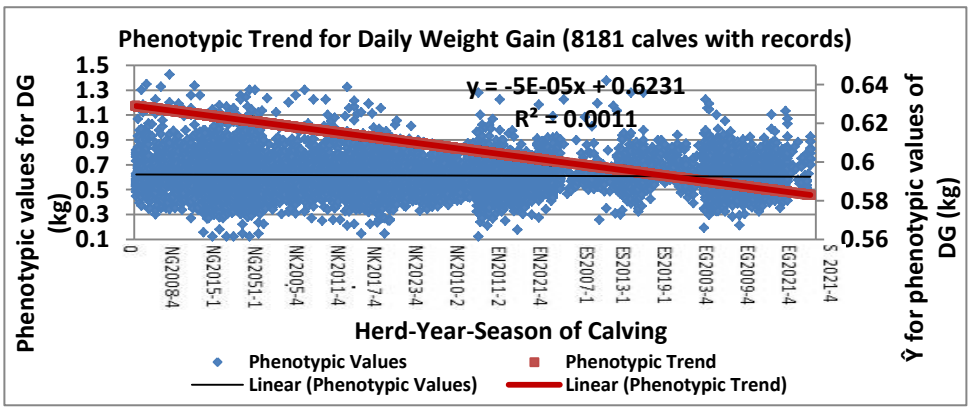


Figure 8. Phenotypic trends for birth weight (BW), weaning weight (WW) and daily weight gain (DG) in Egyptian buffalo plotted by regressing the phenotypic values of growth traits on herd-year season of calving in El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK), El-Nubariya (EN), El-Serw (ES), El-Gimmeza (EG) and Sids (S) herds

4.5 Polymorphic characterization of *PRL* gene

The amplified DNA fragment with a length of 678 *bp* was digested using *XbaI* restriction enzyme to detect the molecular weights of *PRL* gene, where dimorphic genotypes of AA and GG were obtained, while AG genotype was not attained. As shown in **Figures 9** and **10**, the banding patterns of *PRL* gene yielded in PCR product were one band in AA genotype with fragment length of 678 *bp* and two bands in GG genotype with fragment length of 678 and 447 bp*s*. Similarly, **Hasanain *et al*. (2017)** in Egyptian buffalo found that the banding patterns of *PRL* gene were one band of fragment length of 678 *bp* for AA genotype. **Mavi *et al*. (2017)** in Murrah buffalo found one genotype of AA for *PRL* gene with fragment length of 294 bp. **Konca and Akyüz (2017)** reported that the undigested fragment of 156 *bp* for *PRL* gene in Anatolian water buffalo refer to AA genotype, while the fragments of 156, 82 and 74 bp*s* indicated for heterozygous genotype. Also, in Anatolian water buffalo, **Özşensoy (2018)** reported that the undigested fragment of 156 *bp* for *PRL* gene refer to AA genotype, while the fragments of 156, 82 and 74 bp*s* refer to heterozygous genotype.

Across the two studied buffalo herds, as shown in **Table 35**, the genotypic frequency of AA genotype of *PRL* gene was high (0.851) and the frequency of GG genotype was low (0.149). Also in both herds, the allelic frequency recorded for A allele was higher than that recorded for G allele (0.851 *vs* 0.149). In comparing NG herd with NK herd, the frequencies of AA and GG genotypes of *PRL* gene were nearly similar (0.900 *vs* 0.845 for AA genotype; 0.100 *vs* 0.155 for GG genotype). **Ladani *et al*. (2003)** stated that the frequencies of A allele for *PRL* gene in Jaffarabadi, Mehsani and Surti buffaloes were 0.43, 0.50 and 0.48, respectively. **Ishaq *et al*. (2013)** examined the *PRL* gene polymorphisms in Sahiwal and Achai buffalo using PCR-RFLP technique and reported that three genotypes of AA, AG and GG were detected with frequencies of 0.72, 0.18 and 0.10 in Sahiwal buffalo and 0.44, 0.34 and 0.22 in Achai buffalo, respectively. **El-Magd *et al.* (2015)** in Egyptian buffalo found two genotypes for *PRL* gene and reported that the genotypic frequencies were 0.37 for CC genotype and 0.63 for CT genotype

and accordingly the allele frequency was 0.315 for C allele and 0.685 for T allele.

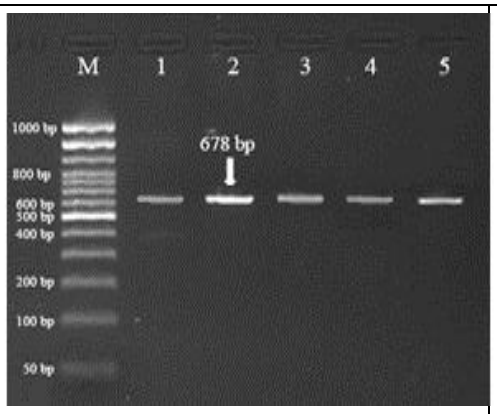


Figure 9. Gel electrophoresis showing the PCR product of *PRL* gene in Egyptian buffalo, 678 *bp* band. M is 50 *bp* ladder DNA molecular marker.

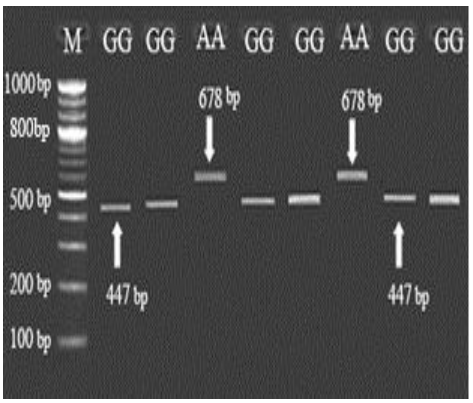


Figure 10. Gel electrophoresis showing the PCR-RFLP of the SNP identified in *PRL* gene in Egyptian buffalo. The genotypes are indicated at the top of each lane, 678 *bp* and 447 *bp* bands, M is 100 *bp* ladder DNA molecular marker.

The effective numbers of alleles (N_e) as an index of genetic diversity revealed that the difference in N_e between NG and NK herds was significant (1.220 vs 1.355, $P<0.01$; **Table 35**). **El-Magd *et al.* (2015)** in Egyptian buffalo. This moderate value $Ne = 1.759$ reflected moderate genetic diversity, polymorphism, and ability to preserve allelic stability after selection or mutation.

Table 35. Molecular characterization parameters for *PRL* gene in NG and NK herds in Egyptian buffalo

Item	NG herd	NK herd	Both herds
Observed number of animals in each *PRL* gene genotype	(N= 30)	(N= 71)	(N= 101)
AA	27	60	86
AG	--	--	--
GG	3	11	15
Expected number of animals in each *PRL* gene genotype	(N= 30)	(N= 71)	(N= 101)
AA	24.3	50.4	73.2
AG	5.4	18.6	25.6
GG	0.3	1.7	2.2
Genotypic frequency:			
AA	0.900	0.845	0.851
AG	--	--	--
GG	0.100	0.155	0.149
Gene frequency:			
A allele	0.900	0.845	0.851
G allele	0.100	0.155	0.149
Effective number of alleles (*Ne*)	1.220^{b}	1.355^{a}	1.339
Chi-square value for Hardy-Weinberg equilibrium ($\chi 2$)	30^{a}	71^{b}	101^{***}
Polymorphic information content (*PIC*)	0.157	0.223	0.211
Observed heterozygosities (H_O)	0.0	0.0	0.0
Expected heterozygosities (H_E)	0.180	0.262	0.253

[a,b] The estimate with the same letters in each column are not significantly different (P≤0.01).

Chi-square values (χ^2) for genotypes of *PRL* gene were highly significant in NG and NK herds (**Table 35**), indicating that both populations were not in Hardy-Weinberg equilibrium *(HWE)* for *PRL* gene, *i.e* degree of variation between the numbers of the expected and observed genotypes was high. This high deviation in *HWE* suggests the change in distribution of alleles from one generation to the next generations. In accordance, **Konca and Akyüz (2017)** showed that the value of Chi-square for genotypes of *PRL* gene in Anatolian water buffalo was high (50.63), indicating that this population was not in *HWE*. The current *PIC* values were low and varied

from 0.157 in NG herd to a moderate value of 0.223 in NK herd and moderate value of 0.211 in both herds (**Table 35**). Depending on the number of detectable alleles and the distribution of their frequency, the value of *PIC* gives an estimate of the marker's discriminating power and, thus, describes the marker's usefulness for identifying the polymorphism within the buffalo population under study **(El-Magd *et al.*, 2015)**. The values of expected heterozygosity (H_E) for *PRL* gene were moderate with values of 0.180 in NG herd, 0.262 in NK and 0.253 in both herds together (**Table 35**). **El-Magd *et al.* (2015)** found that the level of H_E was high (0.431) for *PRL* gene in Egyptian buffalo.

4.6 Polymorphic characterization of *DGAT1* gene

The genotypic frequency of genotype CC was 100% with frequency of 1.0 for allele C and 0.0 for allele T in current herds of Egyptian buffalo. The PCR amplified DNA fragment length of 411 *bp* was digested with *AluI* restriction enzyme and one monomorphic CC genotype of *DGAT1* gene was obtained (**Figures 11** and **12**). *AluI* restriction analysis of the PCR product yielded banding pattern corresponding to one genotype of CC with three bands with fragment length of 176, 167 and 68 bps. **Yuan *et al.* (2007)** in Chinese buffalo reported that the range in band size of *DGAT1* gene was from 160 *bp* to 300 bp. In agreement with the current findings**, Özdil and Ilhan (2012)** in Anatolian buffalo reported that the undigested fragment with 411 *bp* for *DGAT1* gene refer to GG genotype, while the digested fragments of 176, 167 and 68 bps refer to CC genotype and the fragments of 411, 167, 137 and 107 bps were indicated for heterozygous GC genotype. **Freitas *et al.* (2016)** showed that the PCR fragment size was 231 *bp* for *DGAT1* gene in Murrah buffalo. However, *DGAT1* gene is known to control the rate of triglyceride synthesis via adipocytes and consequently could influence the fatty acids composition in milk (**Yuan *et al*., 2007; Tăbăran *et al*., 2015; Liu *et al*., 2020**) and it was verified to have associations with lactation and/or reproduction traits in Chinese buffalo **(Yuan *et al*., 2007)**, in Anatolian buffalo **(Özdil and Ilhan, 2012)**, in Murrah buffalo **(Freitas *et al.*, 2016)**, in

Riverine buffalo **(Li *et al.*, 2017)** and in Egyptian buffalo **(El-Komy *et al.*, 2020)**.

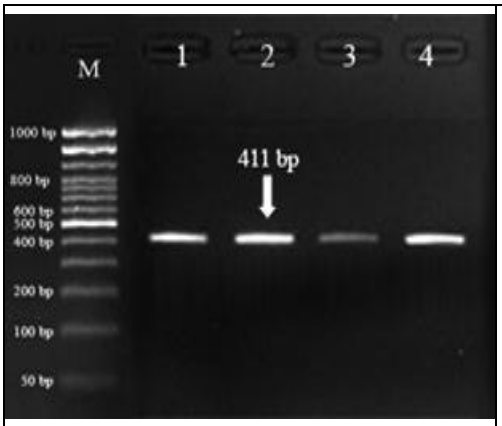


Figure 11. Gel electrophoresis showing the PCR product of *DGAT1* gene in Egyptian buffalo, 411 *bp* band. M is 50 *bp* ladder DNA molecular marker.

Figure 12. Gel electrophoresis showing the PCR-RFLP of the *SNP* identified in *DGAT1* gene in Egyptian buffalo. The genotypes are indicated at the top of each lane. M is 50 *bp* ladder DNA molecular marker**.**

4.7 Polymorphic characterization of *FSHR* gene

The amplified DNA fragment of 306 *bp* was digested using *AluI* restriction enzyme and three genotypes (GG, GC and CC) were obtained for *FSHR* gene in the present study (**Figures 13** and **14**). **Minj *et al.* (2008)** reported that the agarose gel electrophoresis revealed an amplification of 2184 *bp* in Indian River buffalo. As shown in **Figures 13** and **14**, the banding patterns of *FSHR* gene yielded in PCR product were one band in GG genotype (306 bp), two bands in CC genotype (243 and 63 bps) and three bands in GC genotype (306, 243 and 63 bps). **Othman and Abdel-Samad (2013)** in PCR amplified fragments (306 bp) and using *AluI* restriction enzyme in Egyptian buffalo identified three genotypes of *FSHR* gene (CC, CG and GG), indicating that two bands of 243 and 63 bps for CC genotype, three bands of 193, 63 and 50 bps for GG genotype and four bands of 243, 193, 63 and 50 bps for CG genotype. By using the same restriction enzyme

for digestion of 306 *bp* PCR product, **Sosa *et al.* (2015)** differentiated between three genotypes for *FSHR* gene in Egyptian buffalo (CC, TT and CT) and reported that two bands with fragments length of 243 and 63 bps for CC genotype, three bands of 193, 63 and 50 bps for GG genotype and four bands of 243, 193, 63 and 50 bps for CG genotype. **Shafik *et al.* (2017)** showed that one non-synonymous *SNP* (A93G) was detected in Egyptian buffalo in exon 10 of *FSHR* gene with fragment length of 230 bp**.** Recently, **Dhaware *et al*. (2024)** reported that the third fragment of exon 10 of *FSHR* gene was amplified by using forward primer *FSHR3f* and reverse primer *FSHR3r* revealing a PCR product of fragment length 910 *bp* in Indian Marathwadi buffalo.

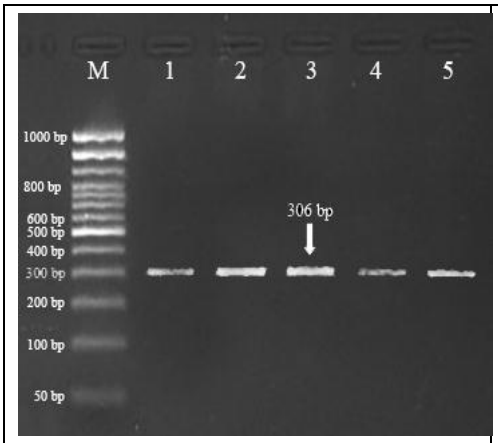


Figure 13. Gel electrophoresis showing the PCR product of *FSHR* gene in Egyptian buffalo, 306 *bp* band. M is 50 *bp* ladder DNA molecular marker.

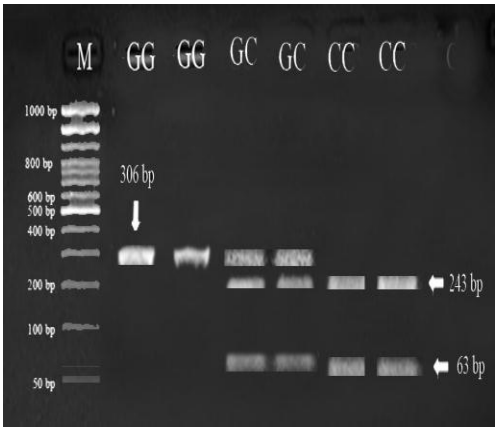


Figure 14. Gel electrophoresis showing the PCR-RFLP of the SNP identified of *FSHR* gene in Egyptian buffalo. The genotypes are indicated at the top of each lane. M is 50 *bp* ladder DNA molecular marker.

Table 36. Molecular characterization parameters for *FSHR* gene in NG, NK and EG herds of Egyptian buffalo

Item	NG herd	NK herd	EG herd	All herds		
				Buffalo cows	Buffalo bulls	Total (cows + bulls)
Observed number of animals in each *FSHR* gene genotype	(N= 33)	(N= 44)	(N= 21)	(N= 98)	(N= 71)	(N= 169)
GG	7	5	2	14	20	34
GC	17	22	11	50	20	70
CC	9	17	8	34	31	65
Expected number of animals in each *FSHR* gene genotype	(N= 33)	(N= 44)	(N= 21)	(N= 98)	(N= 71)	(N= 169)
GG	7.28	5.82	2.68	15.52	12.68	28.17
GC	16.44	20.36	9.64	46.96	34.65	81.66
CC	9.28	17.82	8.68	35.52	23.68	59.17
Genotypic frequency:						
GG	0.212	0.113	0.095	0.14	0.28	0.21
GC	0.515	0.500	0.534	0.51	0.28	0.41
CC	0.272	0.386	0.381	0.35	0.44	0.38
Gene frequency:						
G allele	0.470	0.364	0.357	0.40	0.42	0.408
C allele	0.530	0.636	0.643	0.60	0.58	0.592
Effective number of alleles (*Ne*)	1.993^{ba}	1.862^{c}	1.849^{c}	1.953^{b}	1.920^{a}	1.936^{a}
Chi-square value for *HWE* (χ^2)	0.038^{NS}	0.284^{NS}	0.416^{NS}	0.411^{NS}	12.69^{***}	3.444^{NS}
Polymorphic information content (*PIC*)	0.531	0.653	0.662	0.609	0.586	0.600
Observed heterozygosities (H_O)	0.515	0.500	0.524	0.510	0.282	0.414
Expected heterozygosities (H_E)	0.498	0.463	0.459	0.479	0.488	0.483

[a,b] The estimate with the same letters in each column are not significantly different (P≤0.05); NS= Non-significant (P>0.05).

The genotypic frequencies for genotypes of *FSHR* gene for total buffalo cows and bulls were 0.41 for GC genotype, 0.21 for GG genotype and 0.38 for CC genotype (**Table 36**), *i.e.* allelic frequency for C allele (0.592) was higher than that for G allele (0.408). Also, the genotypic frequency for GG, GC and CC genotypes of *FSHR* gene were 0.14, 0.51 and 0.35 for buffalo cows and 0.28, 0.28 and 0.44 for buffalo bulls. The frequency of GG, GC and CC genotypes of *FSHR* gene in NG, NK and EG herds were widely differed (0.212 in NG herd, 0.113 in NK herd and 0.095 in EG herd for GG genotype; 0.515 in NG herd, 0.500 in NK herd and 0.534 in EG herd for GC genotype; 0.272 in NG herd, 0.386 in NK herd and 0.381 in EG herd for CC genotype). The frequencies for C allele were higher than those for G allele where the frequencies were 0.530, 0.636 and 0.643 for C allele *vs* 0.470, 0.364 and 0.357 for G allele in NG, NK and EG herds, respectively. In Egyptian buffalo, **Shafik *et al.* (2017)** stated that the frequencies for A and G alleles of *FSHR* gene were 0.014 and 0.985 along with frequencies of 0.00, 0.028 and 0.972 for AA, AG and GG genotypes, respectively. **Fouda *et al.* (2021)** reported that the frequencies for C and G alleles were 0.54 and 0.46 with frequencies of 0.34, 0.40 and 0.26 for CC, CG and GG genotypes, respectively.

The difference in the effective number of alleles (N_e) among NG, NK and EG herds for *FSHR* gene were significant ($P<0.01$) where N_e was 1.993, 1.862 and 1.849 in NG, NK and EG herds, respectively (**Table 36**). Also, the N_e were 1.953 and 1.920 in buffalo cows and bulls, respectively. The difference among genotypes of Chi-square for *FSHR* gene were not significant in NG, NK and EG herds (**Table 36**), indicating that all herds were in *HWE* for *FSHR* gene. Similarly, **Fouda *et al.* (2021)** stated that Chi-square values of genotypes (GG and CG) for *FSHR* gene in Egyptian buffalo were moderate (X^2= 3.948 *vs* 7.852), indicating that this population was not in *HWE*. In Indonesian Holstein dairy cattle, **Setyorini *et al.* (2023)** reported that the value of Chi-square for genotypes of *FSHR* gene was high (3.2), *i.e.* *FSHR* gene was not in *HWE* in this population. The current *PIC* values were moderate and varied from 0.531 in NG herd to 0.653 and 0.662 in NK and

EG herds, respectively (**Table 36**). In this respect, **Putra *et al*. (2020)** stated that the PIC values for bovine *FSHR* gene in Indonesian Pasundan cattle were moderate and ranged from 0.30 to 0.50. Moreover, in Zebu × British composite crossbred cattle and indigenous Turkish breed, the PIC values were also moderate, being 0.37 and 0.34, respectively (**Marson *et al*., 2008**; **Arslan *et al*., 2015**). The values of expected heterozygosity (H_E) for *FSHR* gene were high, being 0.498 in NG herd, 0.463 in NK herd, 0.459 in EG herd, 0.479 in buffalo cows and 0.488 in buffalo bulls, while the observed heterozygosities (H_O) were 0.515 in NG herd, 0.500 in NK herd, 0.524 in EG herd, 0.510 in buffalo cows and 0.282 in buffalo bulls (**Table 36**). **Setyorini *et al*. (2023)** in Indonesian Holstein dairy cattle found that the value of *Ho* for *FSHR* gene was 0.490, while the value of *He* was 0.416.

4.8 Polymorphic characterization of *GH* gene

The PCR amplified DNA fragment with length of 211 *bp* was digested using *AluI* restriction enzyme and two genotypes of CC and TC were obtained for *GH* gene (**Figures 15** and **16**). The PCR product using *AluI* restriction enzyme yielded banding pattern corresponding to two bands of 211 and 159 bp*s* for CC genotype and three bands of 211, 159 and 52 bp*s* for TC genotype. Similarly, **Konca and Akyüz (2017)** reported that fragment of 211 *bp* for *GH* gene was observed in Anatolian water buffalo.

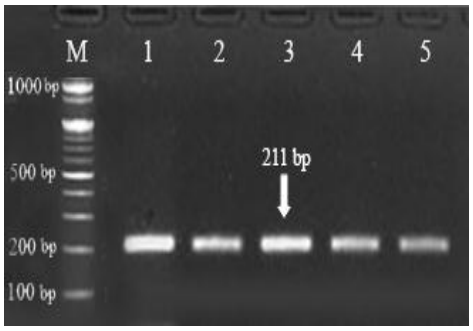


Figure 15. Gel electrophoresis showing the PCR product of *GH* gene in Egyptian buffalo, 211 *bp* band. M is 100 *bp* ladder DNA molecular marker.

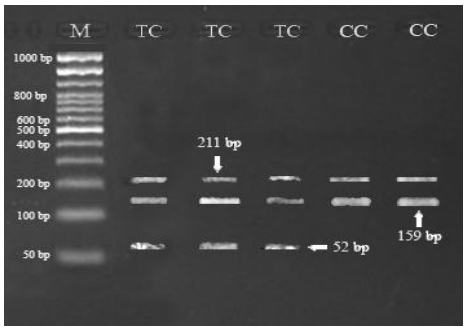


Figure 16. Gel electrophoresis showing the PCR-RFLP of the SNP identified of *GH* gene in Egyptian buffalo. The genotypes are indicated at the top of each lane. M is 50 *bp* ladder DNA molecular marker.

The frequencies of CC genotype for *GH* gene were 0.608 in NG herd, 0.505 in NK herd and 0.500 in EG herd, while the frequencies of TC genotype were 0.392 in NG herd, 0.495 in NK herd and 0.500 in EG herd (**Table 37**). Across all the herds, the frequencies of CC genotype were 0.68 for females, 0.30 for males and 0.52 for both sexes, while the frequencies of TC genotype were 0.32 for females, 0.70 for males and 0.48 for both sexes. The frequencies recorded for C allele (0.804 in NG herd, 0.753 in NK herd and 0.750 in EG herd) were higher than those recorded for T allele (0.196 in NG herd, 0.247 in NK herd and 0.250 in EG herd). **Konca and Akyüz (2017)** reported that the allele frequency in Anatolian buffalo was 0.87 for L allele and 0.13 for V allele, while the frequencies were 0.755, 0.228 and 0.017 for LL, LV and VV genotypes, respectively. **Eriani *et al.* (2019)** stated that the frequency of *GH* gene in Indonesian buffalo was 0.533 for A allele and 0.467 for B allele, with genotypic frequencies of 0.133, 0.866 and 0.066 for AA, AB and BB genotypes, respectively. **Anggraeni *et al.* (2023)** stated that the genotypic frequency of genotypes of *GH* gene in Indonesian Swamp buffalo was 100% for TT genotype and 0% for both TC and CC genotypes, with allelic frequency of 1.0 for T allele and 0.0 for C allele.

Table 37. Molecular characterization parameters for *GH* gene in NG, NK and EG herds of Egyptian buffalo

Item	NG herd	NK herd	EG herd	All herds		
				Females	Males	Total (females + males)
Observed number of animals in each *GH* gene genotype	(N= 51)	(N= 97)	(N= 26)	(N= 103)	(N= 71)	(N= 174)
TT	--	--	--	--	--	--
TC	20	48	13	33	50	83
CC	31	49	13	70	21	91
Expected number of animals in each *GH* gene genotype	(N= 51)	(N= 97)	(N= 26)	(N= 103)	(N= 71)	(N= 174)
TT	1.96	0.594	1.63	2.64	8.80	9.90
TC	16.08	36.12	9.75	27.71	32.39	63.20
CC	32.96	45.94	14.63	72.64	29.80	100.9
Genotypic frequency:						
TT	--	--	--	--	--	--
TC	0.392	0.495	0.500	0.32	0.70	0.48
CC	0.608	0.505	0.500	0.68	0.30	0.52
Gene frequency:						
T allele	0.196	0.247	0.250	0.160	0.352	0.352
C allele	0.804	0.753	0.750	0.840	0.648	0.648
Effective number of alleles (*Ne*)	1.460^{c}	1.593^{b}	1.600^{a}	1.368^{b}	1.839^{a}	1.570
Chi-square value for *HWE* (χ^2)	3.034^{ns}	10.485^{**}	2.889^{ns}	3.748^{ns}	20.97^{***}	17.069^{***}
Polymorphic information content (*PIC*)	0.862	0.801	0.797	0.902	0.668	0.810
Observed heterozygosities (H_O)	0.392	0.495	0.500	0.320	0.704	0.704
Expected heterozygosities (H_E)	0.315	0.372	0.375	0.269	0.456	0.456

[a,b] The estimate with the same letters in each row are not significantly different (P≤0.05); NS= Non-significant (P>0.05), *** = p<0.001

The effective numbers of alleles (N_e) and chi-square values characterizing *GH* gene in each herd are presented in **Table 37**. The difference in allelic numbers among the three herds were significant (*P<0.01*). Across all herds, the highest *Ne* was obtained for males (1.839), while the lowest allelic numbers was obtained for females (1.368). In this regard, **Trakovická *et al.* (2013)** found that N_e for *GH* gene in Slovak Simmental cattle was 1.73. The Chi-square values for genotypes of *GH* gene were not significant in females, however, it was highly significant in males and the total of males and females (**Table 37**), indicating that this buffalo population was in *HWE* for *GH* gene. Similarly, **Konca and Akyüz (2017)** reported that the value of Chi-square for genotypes of *GH* gene was low (0.02), indicating that *GH* gene in Anatolian water buffalo was in *HWE*. **Nafiu *et al.* (2020)** in Swamp buffalo, stated that Chi-square value for genotypes of *GH* gene was also low (0.89), suggesting that the population was in *HWE*. Across the herds and sexes, the values of heterozygosity for *GH* gene were moderate to high and ranged from 0.320 to 0.704 for *Ho* and 0.269 to 0.456 for H_E (**Table 37**). Similarly, **Eriani *et al.* (2019)** in Indonesian buffalo found that the value of *Ho* was high (0.80) and the value of *He* was moderate (0.49). Also, **Nafiu *et al.* (2020)** in Swamp buffalo found that *Ho* value was moderate (0.375), while the value of *He* was high (0.492).

4.9 Polymorphic associations between genotypes of *PRL, FSHR* and *GH* genes and lactation traits or reproduction traits

4.9.1 Molecular associations between genotypes of *PRL* gene and lactation traits or reproduction traits

Two genotypes of AA and GG for *PRL* gene were detected (**Table 38**). However, there were abundant reports evidencing that *PRL* gene is associated with milk production and composition in Pakistan buffalo **(Nadeem and Maryam, 2016)**, in Chinese buffalo **(Li *et al.*, 2017)**, in Turkish buffalo **(Konca and Akyüz., 2017; Özşensoy, 2018)**, in Indian buffalo (**Mavi *et al.*, 2017)** and in Egyptian buffalo (**El-Komy *et al.*, 2020)**. For lactation traits, the GLSM for SNP genotypes of *PRL* gene showed that

there were molecular associations of AA and GG genotypes with test-day milk traits (**Table 38**).

The differences in GLSM for lactation traits between AA and GG genotypes of *PRL* gene in NG and NK herds were significantly in favour of AA genotype (*P<0.01*, **Table 38**). In both NG and NK herds, high GLSM were recorded for AA genotype to be 6.0 kg for TDMY, 390 g for TDFY, 290 g for TDPY and 2.47 log^{10} for TDSCS compared with 5.3 kg, 340 g, 220 g and 2.50 log^{10} for GG genotype, respectively. In NG herd, GLSM for lactation traits were significantly in favour of AA genotype of *PRL* gene relative to GG genotype in terms of 5.9 *vs* 5.5 kg for TDMY, 360 *vs* 310 g for TDFY, 260 *vs* 220 g for TDPY and 2.38 *vs* 2.52 log^{10} for TDSCS, while the respective GLSM in NK herd were 5.97 *vs* 5.43 kg, 390 *vs* 350 g, 290 *vs* 230 g and 2.41 *vs* 2.49 log^{10}. *PRL* hormone has several biological functions related to reproduction, osmoregulation, integument growth, and synergism with steroids because the *PRL* gene is expressed in the pituitary gland as well as at various other locations, such as the mammary gland, the central nervous system, and the immune system (**Le Provost *et al*., 1994 and Sinha, 1995**). **Lazebnaya *et al*. (2013)** reported that the interaction of bovine *PRL* gene and its receptor (*PRLR*) following its expression starts a signalling cascade that triggers the transcription of other several genes, including those pertaining to milk proteins such as caseins and lactalbumin. However, *PRL* gene is known to have various biological functions such as water and electrolyte balance, growth and development, immune and reproduction function (**Freeman *et al*., 2000; Singh *et al*., 2015**). Also, *PRL* gene plays a central role in mammalian reproduction, glandular development, milk secretion, and expression of milk protein. In Murrah buffalo, **Singh *et al*. (2015)** found that *PRL* gene is an important candidate gene known to be associated with milk production traits as well as somatic cell score (SCS).

Table 38. Molecular associations between genotypes of *PRL* gene (AA and GG genotypes) and test-day lactation traits or reproduction performance expressed as generalized least square means and their standard errors (GLSM±SE)

Herd and lactation trait	AA Genotype		GG Genotype		Herd and reproduction trait	AA Genotype		GG Genotype	
	GLSM	SE	GLSM	SE		GLSM	SE	GLSM	SE
NG herd:	**(N= 282)**		**(N= 42)**		**NG herd:**	**(N= 125)**		**(N= 18)**	
TDMY(kg)	5.9[a]	0.09	5.5[b]	0.25	AFC (*mo*)	43.0[a]	0.4	41.0[b]	1.2
TDFY (kg)	0.36[a]	0.01	0.31[b]	0.02	DO (*d*)	174[a]	9.4	142[b]	24.9
TDPY (kg)	0.26[a]	0.04	0.22[b]	0.01	CI (*d*)	476[a]	9.4	435[b]	24.8
TDSCS (log^{10})	2.38[b]	0.03	2.52[a]	0.01					
NK herd:	**(N= 590)**		**(N= 115)**		**NK herd:**	**(N=162)**		**(N=48)**	
TDMY(kg)	5.97[a]	0.09	5.43[b]	0.20	AFC (*mo*)	35.1[a]	0.3	33.8[b]	0.6
TDFY (kg)	0.39[a]	0.01	0.35[b]	0.01	DO (*d*)	158[a]	8.1	143[b]	14.9
TDPY (kg)	0.29[a]	0.04	0.23[b]	0.04	CI (*d*)	459[a]	7.9	449[b]	14.6
TDSCS (log^{10})	2.41[b]	0.03	2.49[a]	0.08					
Both herds:	**(N= 872)**		**(N= 157)**		**EG herd:**	**(N=82)**		**(N=18)**	
TDMY(kg)	6.0[a]	0.06	5.3[b]	0.15	AFC (*mo*)	37.4[a]	0.4	34.8[b]	0.9
TDFY (kg)	0.39[a]	0.01	0.34[b]	0.01	DO (*d*)	185[a]	23.0	170[b]	11.6
TDPY (kg)	0.29[a]	0.00	0.22[b]	0.01	CI (*d*)	481[a]	23.1	469[b]	11.6
TDSCS (log^{10})	2.47[b]	0.01	2.50[a]	0.02					
					All herds:	**(N=369)**		**(N=84)**	
					AFC (*mo*)	37.4[a]	0.2	36.5[b]	0.6
					DO (*d*)	166[a]	5.3	154[b]	11.2
					CI (*d*)	467[a]	5.3	455[b]	11.1

N= Number of test-day lactation records or number of reproduction records.

[a,b] GLSM within each classification, not followed by the same letter in the row differed significantly (*P<0.01*).

For most reproduction traits, GLSM for AA and GG genotypes of *PRL* gene showed that there were significant molecular associations between AA and GG genotypes with AFC, DO and CI (**Table 38**). Also, the differences in GLSM for AFC, DO and CI between AA and GG genotypes of *PRL* gene were significantly in favour of GG genotype relative to AA genotype in NG, NK and EG herds (*P<0.01*), *i.e.* GLSM for GG genotype ranging from 33.8 to 41 *mo* for AFC, 142 to 170 *d* for DO and 435 to 469 *d* for CI. In NG herd, GLSM for GG genotype of *PRL* gene were significantly

favourable for reproduction traits compared to AA genotype in terms of 41.0 *vs* 43.0 *mo* for AFC, 142 *vs* 174 *d* for DO and 345 *vs* 476 *d* for CI, while the corresponding GLSM in NK were 33.8 *vs* 35.1 *mo*, 143 *vs* 158 *d* and 449 *vs* 459 *d*.

4.9.2 Molecular associations between genotypes of *FSHR* gene and lactation traits or reproduction traits

Follicle stimulating hormone drives the growth, differentiation, maturity, and ovulation of ovarian follicles by attaching this gene to its receptor (*FSHR*) on the surface of the ovary (**Chu and Majumdar, 2012**). For polymorphism of genes related to reproduction traits in buffalo, *FSHR* gene is essential for follicle growth, development, differentiation, triggering the maturation and ovulation of ovarian follicles. **Dhaware *et al.* (2024)** stated that *FSHR* plays a critical role in the development of anatomical, functional, and behavioral qualities required for buffalo reproduction.

The differences in GLSM for lactation traits among GG, GC and CC genotypes of *FSHR* gene in the three herds studied were significantly in favour of CC genotype (*P<0.01*, **Table 39**). The GLSM in NG herd were significantly in favour of CC genotype of *FSHR* gene relative to GC and GG genotypes in terms of 6.8 kg *vs* 5.7 and 6.0 kg for TDMY, 480 g *vs* 380 and 410 g for TDFY, 280 g *vs* 220 and 250 g for TDPY and 2.41 $\log^{10}$ *vs* 2.43 and 2.49 $\log^{10}$ for TDSCS, while the corresponding GLSM in NK herd were 6.8 kg *vs* 5.4 and 5.5 kg, 390 g *vs* 340 and 320 g, 290 g *vs* 250 and 230 g and 2.41 $\log^{10}$ *vs* 2.45 and 2.49 $\log^{10}$. **Shafik *et al.* (2017)** reported significant association between *FSHR* gene and total milk yield and 305-day milk yield in Egyptian buffalo.

Table 39. Molecular associations between genotypes of *FSHR* gene (GG, GC and CC genotypes) and lactation traits expressed as generalized least square means and their standard errors (GLSM±SE)

Herd and lactation trait	GG Genotype		GC Genotype		CC Genotype	
	GLSM	SE	GLSM	SE	GLSM	SE
NG herd:	**(N= 107)**		**(N= 212)**		**(N= 116)**	
TDMY (kg)	6.0[b]	0.186	5.7[c]	0.132	6.8[a]	0.179
TDFY (kg)	0.41[c]	0.016	0.38[b]	0.012	0.48[a]	0.015
TDPY (kg)	0.25[b]	0.008	0.22[b]	0.005	0.28[a]	0.007
TDSCS (log^{10})	2.49[a]	0.026	2.43[b]	0.019	2.41[b]	0.025
NK herd:	**(N= 87)**		**(N= 392)**		**(N= 288)**	
TDMY (kg)	5.5[b]	0.232	5.4[a]	0.109	6.8[a]	0.127
TDFY (kg)	0.32[b]	0.016	0.34[b]	0.007	0.39[a]	0.009
TDPY (kg)	0.23[c]	0.010	0.25[b]	0.005	0.29[a]	0.006
TDSCS (log^{10})	2.49[a]	0.025	2.45[b]	0.012	2.41[c]	0.014
Both herds:	**(N= 194)**		**(N= 604)**		**(N= 404)**	
TDMY (kg)	5.6[b]	0.139	5.7[b]	0.078	6.8[a]	0.096
TDFY (kg)	0.36[a]	0.007	0.35[b]	0.006	0.38[a]	0.010
TDPY (kg)	0.22[c]	0.006	0.23[b]	0.003	0.29[a]	0.004
TDSCS (log^{10})	2.48[a]	0.017	2.45[b]	0.010	2.41[c]	0.012

[a,b] GLSM within each classification, not followed by the same letter in the row differed significantly (*P<0.01*).

The differences in GLSM for reproduction traits among GG, GC and CC genotypes of *FSHR* gene were significantly in favour of CC genotype (*P<0.01*, **Table 40**). The GLSM recorded for CC genotype of *FSHR* gene were significantly favourable lower than GLSM for GC and GG genotypes in terms of 37.9 *mo vs* 39.7 and 42.5 *mo* for AFC, 83 *d vs* 91 and 102 *d* for DO and 387 *d vs* 397 and 419 *d* for CI in NG herds, comparable with 32.0 *mo vs* 34.3 and 35.6 *mo* for AFC, 83 *d vs* 91 and 102 *d* for DO and 384 *d vs* 408 and 398 *d* for CI in NK herd (**Table 40**). Also, favourable trends were observed in EG herd where GLSM were 35.0 *mo vs* 37.3 and 36.5 *mo* for AFC, 103 *d vs* 109 and 118 *d* for DO and 366 *d vs* 396 and 410 *d* for CI. Several Egyptian studies have shown that *FSHR* gene is considered as an important candidate gene for reproduction and fertility traits in Egyptian buffalo (**Othman and**

Abdel-samad, 2013; Shafik *et al*., 2017; Ramadan *et al*., 2020; Fouda *et al*., 2021; Sallam *et al*., 2022).

Table 40. Molecular associations between genotypes of *FSHR* gene (GG, GC and GC genotypes) and reproduction traits expressed as generalized least square means and their standard errors (GLSM±SE)

Herd and reproduction trait	GG Genotype		GC Genotype		CC Genotype	
	GLSM	SE	GLSM	SE	GLSM	SE
NG herd:	**(N=37)**		**(N=76)**		**(N=38)**	
AFC (*mo*)	42.5[a]	0.78	39.7[b]	0.51	37.9[c]	0.73
DO (*d*)	102[a]	13.9	91[b]	9.7	83[c]	13.8
CI (*d*)	419[a]	15.3	397[b]	10.7	387[c]	15.16
NK herd:	**(N=28)**		**(N=114)**		**(N=80)**	
AFC (*mo*)	35.6[a]	0.85	34.3[b]	0.42	32.0[c]	0.50
DO (*d*)	102[a]	14.7	91[b]	7.3	83[c]	8.7
CI (*d*)	398[b]	15.9	408[a]	7.9	384[c]	9.4
EG herd:	**(N=9)**		**(N=39)**		**(N=36)**	
AFC (*mo*)	36.5[b]	1.36	37.3[a]	0.65	35.0[c]	0.68
DO (*d*)	118[a]	26.4	109[b]	12.7	103[c]	13.2
CI (*d*)	410[a]	29.1	396[b]	14.0	366[c]	14.5
All herds:	**(N=74)**		**(N=240)**		**(N=154)**	
AFC (*mo*)	38.4[a]	0.61	37.6[b]	0.33	34.5[c]	0.42
DO (*d*)	115[a]	9.1	108[b]	5.0	100[c]	6.3
CI (*d*)	411[a]	9.8	402[b]	5.4	391[c]	6.8

[a,b] GLSM within each classification, not followed by the same letter in the row differed significantly (*P<0.01*).

4.9.3 Molecular associations between genotypes of *GH* gene and lactation traits or reproduction traits:

The *GH* gene is thought to be a positional and functional candidate gene for ruminant qualities that have economic significance, like growth, carcass, and milk features (**Sejrsen *et al*., 1999**). This gene produces the anabolic hormone *GH* protein, which is produced by the anterior pituitary's somatotrophic cells (**Ayuk and Sheppard, 2006**). *GHR*, the receptor for growth hormone, is expressed in a number of organs, most notably the

muscles, adipose tissues, and liver and growth hormone acts by binding to this receptor (**Sellier, 2000**).

Two genotypes of TC and CC for *GH* gene in each separate NG and NK herds were significantly in favour of TC genotype for lactation traits (*P<0.01,* **Table 41**). The GLSM for TC genotype of *GH* gene in NG herd were significantly higher in lactation traits than that of CC genotype in terms of 6.3 *vs* 5.8 kg for TDMY, 480 *vs* 380 g for TDFY and 290 *vs* 230 g for TDPY (**Table 41**). Also, favourable respective GLSM of 6.3 *vs* 5.6 kg, 390 *vs* 350 g and 290 *vs* 230 g were confirmed in NK herd. Moreover, GLSM of TDSCS were in favour of TC genotype relative to CC genotype (2.41 and 2.45 ^{log10} in NG and NK herds). Furthermore, *GH* gene can be used as a candidate gene in the genetic improvement programs for growth traits in buffalo, since this gene is known to have various biological functions such as water and electrolyte balance and milk production (**El-Komy *et al*., 2020** and **Othman *et al*., 2011b**). Growth hormone, as a key component of the somatotrophic axis, is essential for growth, reproduction, and breast feeding primarily via promoting cell division, the synthesis of proteins and lipids, and metabolism (**Davis *et al*., 2021**).

Table 41. Molecular associations between genotypes of *GH* gene (TC and CC genotypes) and lactation and reproduction traits expressed as generalized least square means and their standard errors (GLSM±SE)

Herd and lactation trait	TC Genotype		CC Genotype		Herd and reproduction trait	TC Genotype		CC Genotype	
	GLSM	SE	GLSM	SE		GLSM	SE	GLSM	SE
NG herd:	**(N= 98)**		**(N= 355)**		**NG herd:**	**(N=106)**		**(N=37)**	
TDMY(kg)	6.3[a]	0.19	5.8[b]	0.10	AFC (*mo*)	37.8[b]	0.79	41.4[a]	0.47
TDFY (kg)	0.48[a]	0.02	0.38[b]	0.08	DO (*d*)	93[b]	14.1	115[a]	8.3
TDPY (kg)	0.29[a]	0.01	0.23[b]	0.04	CI (*d*)	383[b]	15.6	407[a]	9.2
TDSCS (log^{10})	2.41[b]	0.03	2.45[a]	0.01					
NK herd:	**(N= 297)**		**(N= 481)**		**NK herd:**	**(N=154)**		**(N=70)**	
TDMY(kg)	6.3[a]	0.12	5.6[b]	0.09	AFC (*mo*)	33.7[b]	0.54	35.2[a]	0.36
TDFY (kg)	0.39[a]	0.01	0.35[b]	0.01	DO (*d*)	94[b]	9.4	100[a]	6.3
TDPY (kg)	0.29[a]	0.01	0.23[b]	0.04	CI (*d*)	379[b]	10.2	393[a]	6.9
TDSCS (log^{10})	2.41[b]	0.01	2.45[b]	0.01					
Both herds:	**(N= 395)**		**(N= 836)**		**EG herd:**	**(N=59)**		**(N=34)**	
TDMY(kg)	6.1[a]	0.10	5.6[b]	0.07	AFC (*mo*)	35.4[b]	0.65	37.5[a]	0.49
TDFY (kg)	0.39[a]	0.01	0.35[b]	0.00	DO (*d*)	105[b]	13.4	121[a]	10.2
TDPY (kg)	0.29[a]	0.04	0.22[b]	0.00	CI (*d*)	395[b]	15.0	406[a]	11.4
TDSCS (log^{10})	2.41[b]	0.01	2.45[a]	0.01					
					All herds:	**(N=326)**		**(N=145)**	
					AFC (*mo*)	34.4[b]	0.44	37.6[a]	0.29
					DO (*d*)	95[b]	6.7	107[a]	4.5
					CI (*d*)	377[b]	7.4	399[a]	4.9

N= Number of test-day lactation records or Number of reproduction records.

[a,b] GLSM within each classification, not followed by the same letter in the row differed significantly ($P<0.01$).

For the molecular association between the genotypes of *GH* gene with reproduction traits in NG, NK and EG herds, the differences between TC and CC genotypes were significantly in favour of TC genotype (**Table 41**). The GLSM recorded for TC genotype in NG, NK and EG herds were significantly the lowest favourable genotypes for AFC (37.8, 33.7 and 35.4

mo), DO (93, 94 and 105 *d*) and CI (383, 379 and 395 *d*), comparable with the corresponding GLSM of CC genotype (41.4, 35.2 and 37.5 *mo* for AFC, 115, 100 and 121 *d* for DO and 407, 393 and 406 *d* for CI).

4.10 Molecular associations between *FSHR* gene *or GH* gene and semen traits

The differences in GLSM for semen traits among GG, GC and CC genotypes of *FSHR* gene were significantly in favour of GG genotype relative to GC and CC genotypes in terms of 2.9 *ml vs* 2.6 and 2.5 *ml* for EV trait, 64.1 *% vs* 59.3 and 63.2 *%* for MS trait, 62.8 *% vs* 57.8 and 61.9 *%* for LS trait, 9.1*% vs* 9.9 and 9.4*%* for AS trait, 1.59×10^9 sperms per ml *vs* 1.36 and 1.50×10^9 sperms per ml for SC trait (*P<0.01*, **Table 42**). **Sallam *et al.* (2022)** reported significant association between *FSHR* gene and sperm motility in Egyptian buffalo. **Sang *et al.* (2011)** reported significant association between *FSHR* gene and EV and SC in Chinese Holstein cattle. Also, **Nikitkina *et al.* (2021)** showed that the associations between *FSHR* gene and semen quality traits were significant (*P<0.05*) for EV and SC and non-significant for MS trait. Recently, **Khan *et al.* (2024)** reported that *FSHR* and other genes such as *INHA*, *INHAB*, *TNP2* and *SPEF2* were detected to be involved with sperm structural integrity, cellular communication, and DNA repair, all of which are critical for spermatogenesis and sperm function. The relationship between *FSHR* gene and the enhancement in semen traits in dairy bulls was previously explained by **Yang *et al.* (2013)** who reported that a mutation in the SNP in the five upstream regions of the bovine *FSHR* gene may have changed the transcription-factor binding sites, which in turn may have changed the expression of the *FSHR* gene by affecting spermatogenesis in the testis and changing gene expression in the Sertoli cells. Concurrently, the favourable impacts on sperm concentration and ejaculate volume may be accounted by the favourable genetic association between these traits.

Table 42. Molecular associations between *FSHR* gene or *GH* gene and semen traits in Egyptian buffalo expressed as generalized least square means and their standard errors (GLSM±SE) estimated by PEST software

Semen traits	*FSHR* gene						*GH* gene			
	Number of bulls									
	GG (N= 21)		GC (N= 20)		CC (N= 31)		TC (N=50)		CC (N=21)	
	GLSM	SE	GLSM	SE	GLSM	SE	GLSM	SE	GLSM	SE
	Number of ejaculates									
	N=189		N=245		N=279		N=450		N=188	
EV (*ml*)	2.9^{a}	0.08	2.6^{b}	0.07	2.5^{b}	0.07	2.5^{b}	0.06	2.9^{a}	0.04
MS (%)	64.1^{a}	0.91	59.3^{c}	0.75	63.2^{b}	0.81	60.9^{b}	0.58	64.1^{a}	0.92
LS (%)	62.8^{a}	0.90	57.8^{c}	0.74	61.9^{b}	0.79	60.0^{b}	0.58	62.1^{a}	0.91
AS (%)	9.1^{c}	0.29	9.9^{a}	0.24	9.4^{b}	0.25	9.6^{a}	0.18	8.9^{b}	0.28
SC (10^9 sperms per ml)	1.59^{a}	0.04	1.36^{c}	0.34	1.50^{b}	0.32	1.4^{b}	0.03	1.6^{a}	0.04

N= Number of semen traits records; EV= Ejaculate volume; MS=Motility sperm; LS= Live sperm; AS=Abnormal sperm; SC= Sperm concentration.

^{a,b} GLSM within each classification, not followed by the same letter in the row differed significantly (*P<0.01*).

The molecular association analyses for semen traits revealed that two genotypes of TC and CC were detected (Dimorphic) for *GH* gene (**Table 42**). The associations were significantly in favour of CC genotype relative to TC genotype (*P<0.01*). The GLSM for semen traits were significantly in favour of CC genotype compared to TC genotype (2.9 *ml vs* 2.5 *ml* for EV trait; 64.1 *% vs* 60.9 *%* for MS trait; 62.1 *% vs* 60.0 *%* for LS trait; 8.9*% vs* 9.6 *%* for AS trait; 1.60×10^9 sperm per *ml vs* 1.40×10^9 sperm per *ml* for SC trait). **Darwish *et al* (2016)** found that there were significant positive associations (*P<0.05*) of LV genotype for *GH* gene with EV and MS traits in Egyptian buffalo. In dairy and beef bulls, **Lechniak *et al*. (1999)** indicated that variations in *GH* gene genotypes may have an impact on the pattern of sperm production in bulls. In Holstein bulls, **Afshar *et al*. (2010)** showed also that there were significant associations between genotypes of *GH* gene and semen traits, since LL genotype was the lowest in EV trait, while VV genotype was

the highest in LS and SC traits. Moreover, **Pal *et al.* (2014)** with two genotypes for *GH* gene (LL and LV) in a crossbred between Holstein Friesian and local Indian Tharparkar cattle, reported that LL genotype was positively associated with sperms motility, live sperm count, acrosomal integrity, hypo-osmotic swelling test (HOST), and number of semen doses per collection.

4.11 Molecular associations between *GH* gene or *PRL* gene and growth traits

The molecular association analyses for growth traits revealed that two genotypes (Dimorphic) of TC and CC were detected for *GH* gene in each separate herd. The associations were significantly in favour of TC genotype in NG, NK and EG herds (*P<0.01*, **Table 43**). Across all herds, GLSM for TC genotype had significantly heavier body weights and gains than CC genotype (36.8 *vs* 33.9 *kg* for BW, 96.3 *vs* 91.8 *kg* for WW and 600 *vs* 540 *g* for DG). For each experimental herd, the GLSM of BW, WW and DG of TC genotype were favorably higher relative to CC genotype (36.9 *kg*, 94.8 *kg* and 590 *g vs* 34.4 *kg*, 91.2 *kg* and 560 *g* in NG herd; 38.0 *kg*, 95.9 *kg* and 580 *g vs* 36.5 *kg*, 90.9 *kg* and 530 *g* in NK herd; 39.0 *kg*, 104.6 *kg* and 660 *g vs* 33.0 *kg*, 91.5 *kg* and 530 *g* in EG herd). The Egyptian buffalo studies have shown that *GH* gene can be used as a candidate gene for the genetic improvement of growth traits (**Othman *et al*., 2012a; Darwish *et al*., 2016**). Also, the non-Egyptian buffalo studies have shown polymorphic associations between *GH* candidate gene and growth and carcass traits in Indonesian buffalo (**Andreas *et al*., 2010; Eriani *et al*., 2019; Nafiu *et al*., 2020)** and Anatolian buffalo in Turkey **(Konca and Akyüz, 2017; Özkan Ünal *et al*., 2020**).

Table 43. Molecular associations between *GH* gene or *PRL* gene and growth traits in Egyptian buffalo expressed as generalized least square means and their standard errors (GLSM±SE)

Herd and growth trait	*GH* gene				Herd and growth trait	*PRL* gene			
	TC Genotype		CC Genotype			AA Genotype		GG Genotype	
	GLSM	SE	GLSM	SE		GLSM	SE	GLSM	SE
NG herd (N=51):	**(N=20)**		**(N=31)**		**NG herd (N=33):**	**(N=29)**		**(N=4)**	
BW (*kg*)	36.9[a]	1.3	34.4[b]	1.08	BW (kg)	43.9[a]	1.36	33.9[b]	3.67
WW(*kg*)	94.8[a]	2.2	91.2[b]	1.80	WW(kg)	95.9[a]	1.96	92.6[b]	5.28
DG (*g*)	590[a]	12	560[b]	22	DG (g)	594[a]	26	472[b]	71
NK herd (N=70):	**(N=23)**		**(N=47)**		**NK herd (N=45):**	**(N=37)**		**(N=8)**	
BW (*kg*)	38.0[a]	0.6	36.5b	0.67	BW (kg)	36.4[a]	0.90	34.0[b]	1.94
WW(*kg*)	95.9[a]	1.6	90.9[b]	1.62	WW(kg)	95.0[a]	1.85	90.8[b]	3.97
DG (*g*)	580[a]	20	530[b]	19	DG (g)	605[a]	19	542[b]	40
EG herd (N=53):	**(N=40)**		**(N=13)**		**EG herd (N=23):**	**(N=20)**		**(N=3)**	
BW (*kg*)	39.0[a]	1.3	33.0[b]	1.31	BW (kg)	43.7[a]	0.99	34.8[b]	2.56
WW(*kg*)	104.6[a]	3.3	91.5[b]	3.27	WW(kg)	99.9[a]	2.66	77.6[b]	6.87
DG (*g*)	660[a]	20	530[b]	34	DG (g)	623[a]	26	367[b]	68
All herds (N=174):	**(N=83)**		**(N=91)**		**All herds (N=101):**	**(N=86)**		**(N=15)**	
BW (*kg*)	36.8[a]	0.5	33.9[b]	0.53	BW (kg)	38.6[a]	0.63	36.1[b]	1.52
WW(*kg*)	96.3[a]	1.2	91.8[b]	1.18	WW(kg)	93.7[a]	1.2	90.8[b]	2.93
DG (*g*)	600[a]	12	540[b]	13	DG (g)	594[a]	13	568[b]	32

N= Number of calves; BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.

[a,b] GLSM within each classification, not followed by the same letter in the row differed significantly (*P<0.01*)

Regarding the *PRL* gene, the molecular association analyses showed that two genotypes of AA and GG for *PRL* gene (Dimorphic) were identified in each herd (NG, NK and EG; **Table 43**). Across all herds, GLSM for AA genotype were significantly heavier in body weights and gains (*P<0.01*) than GG genotype (38.6 *vs* 36.1 *kg* for BW, 93.7 *vs* 90.8 *kg* for WW and 594 *vs* 568 *g* for DG). Similarly, the GLSM in each separate herd for BW, WW and DG in AA genotype were favorably higher in weights and gains relative to GG genotype (43.9 *kg*, 95.9 *kg* and 594 *g vs* 33.9 *kg*, 92.6 *kg* and 472 *g* in NG herd; 36.4 *kg*, 95.0 *kg* and 605 *g vs* 34.0 *kg*, 90.8 *kg* and 542 *g* in NK herd;

43.7 *kg*, 99.9 *kg* and 623 *g vs* 34.8 *kg*, 77.6 *kg* and 367 *g* in EG herd). To our knowledge, there are no previous studies concerning the molecular association between *PRL* gene and growth traits in buffalo although there are limited studies available in cattle. **Meyer *et al*. (2017)** demonstrated that genotypes of *PRL* gene impacted significantly heavier live body weights of Angus calves at birth and weaning.

4.12 Molecular associations between *FSHR* gene and growth traits

The molecular association analyses revealed that three genotypes (Polymorphic) of GG, GC and CC for *FSHR* gene were detected and the differences in GLSM among these genotypes for BW, WW and DG were significantly in favour of GG relative to GC and CC genotypes (*P<0.01*; **Table 44**). Across all herds, the GLSM for GG genotype were significantly heavier in weights than GC and CC genotypes (38.8 *kg vs* 35.4 and 36.8 *kg* for BW; 97.9 *kg vs* 92.8 and 92.6 *kg* for WW; 603 *g vs* 558 and 452 *g* for DG). In each of NG and NK herds, GLSM of GG genotype for BW, WW and DG were favorably heavier in weighs and gains relative to GC and CC genotype (37.7 *kg*, 102.9 *kg* and 662 *g* for GG genotype *vs* 32.9 *kg*, 89.1 *kg* and 550 *g* for GC genotype and 36.3 *kg*, 88.8 *kg* and 524 *g* for CC genotype in NG herd; 38.1 *kg*, 95.3 *kg* and 577 *g* for GG genotype *vs* 36.5 *kg*, 92.8 *kg* and 552 *g* for GC genotype and 36.5 *kg*, 92.5 *kg* and 537 *g* for CC genotype in NK herd). Oppositely, GLSM for BW, WW and DG of CC genotype in EG herd were significantly heavier than GG and GC genotypes (39.4 *kg*, 106.2 *kg* and 636 *g* for CC genotype*vs* 33.3 *kg*, 93.3 *kg* and 574 *g* for GC genotype and 35.5 *kg*, 92.5 *kg* and 548 *g* for GG genotype). The reverse trend of association between the SNP genotypes of *FSHR* gene and growth traits obtained in EG herd could be attributable to the small number of the genotyped animals (24 calves; **Table 44**) compared with the other two herds (47 calves in NG and 101 calves in NK herd).

The differences in GLSM among GG, GC and CC genotypes of *FSHR* gene for BW, WW and DG were significantly in favour of GG relative to GC and CC genotypes of NK herd). Therefore, more genotyped animals are required to represent the sample of the buffalo population raised in the EG

herd. To our knowledge, no anterior studies have been detected either on buffalo or cattle for assessing the molecular relationship between the *FSHR* gene and growth traits.

Table 44. Molecular associations between *FSHR* gene and growth traits in Egyptian buffalo expressed as generalized least square means and their standard errors (GLSM±SE)

Herd and growth trait	GG Genotype		GC Genotype		CC Genotype	
	GLSM	SE	GLSM	SE	GLSM	SE
NG herd (N=47)	**(N= 10)**		**(N= 23)**		**(N= 14)**	
BW (*kg*)	37.7^{a}	1.92	32.9^{c}	1.27	36.3^{b}	1.63
WW (*kg*)	102.9^{a}	3.21	89.1^{b}	2.12	88.8^{b}	2.71
DG (*g*)	662^{a}	39	550^{b}	25	524^{b}	33
NK herd (N=101)	**(N= 22)**		**(N= 38)**		**(N= 41)**	
BW (*kg*)	38.1^{a}	0.85	36.5^{b}	0.65	36.5^{b}	0.62
WW (*kg*)	95.3^{a}	2.83	92.8^{b}	1.90	92.5^{b}	1.83
DG (*g*)	577^{a}	28	552^{a}	212	537^{a}	20
EG herd (N=24)	**(N= 3)**		**(N= 11)**		**(N= 10)**	
BW (*kg*)	36.5^{b}	2.67	33.3^{c}	1.39	39.4^{a}	1.46
WW (*kg*)	92.5^{b}	6.42	93.3^{b}	3.35	106.2^{a}	3.52
DG (*g*)	548^{b}	69	574^{b}	36	636^{a}	37
All herds (N=172)	**(N= 35)**		**(N= 72)**		**(N= 65)**	
BW (*kg*)	**38.8^{a}**	**0.55**	**35.4^{c}**	**0.79**	**36.8^{b}**	**0.57**
WW (*kg*)	97.9^{a}	1.93	92.8^{b}	1.35	92.6^{b}	1.40
DG (*g*)	603^{a}	21	558^{b}	151	452^{b}	15

N= Number of growth traits records; BW= Birth weight; WW=Weaning weight; DG= Daily weight gain.

^{a,b} GLSM within each classification, not followed by the same letter in the row differed significantly ($P<0.01$).

5. SUMMARY

The main objectives of the present study were: 1) to evaluate genetically some lactation, reproduction, semen and growth traits in some Egyptian buffalo herds through estimating the variance components and heritability using Bayesian Gibbs Sampling Algorithm applying single trait animal model and random regression model (RRM), 2) to predict the breeding values (PBVs) and plot the genetic and phenotypic trends for these traits using BLUPF90 software, 3) to characterize on *SNPs* basis some candidate genes of *PRL*, *DGAT1*, *FSHR* and *GH* in Egyptian buffalo, 4) to use PCR-RFLP technique in genotyping the *SNP* genotypes located in the promoter regions of these genes, and 5) to detect the molecular associations of *SNP* genotypes of *PRL*, *FSHR* and *GH* candidate genes with milk production and composition, reproduction, semen and body weight traits in Egyptian buffalo using generalized least square means procedure (GLSM).

For quantitative genetic analyses in this study, four sets of data in terms of lactation, reproduction, semen and body weights were used. A pedigreed file of 7345 Test-Day (TD) records of milk (TDMY), fat (TDFY), protein (TDPY) yields and somatic cell scores (TDSCS) were gathered monthly from 686 buffaloes, daughters of 83 sires and 423 dams for a period of 21 years starting from 2003 up to 2023 in three experimental buffalo herds of El-Nattafe El-Gadid (NG), El-Nattafe El-Kadim (NK) and El-Gimmeza (EG). Also, a total number of 7279 reproduction records of age at first calving (AFC), days open (DO) and calving interval (CI)) were collected for a period of 22 years (2002 to 2023) from 1951 buffaloes, daughters of 155 sires and 1179 dams in six experimental herds of NG, NK, EG, El-Nubariya (EN), El-Serw (ES) and Sids (S). Data of 5178 semen ejaculates were collected from 111 Egyptian buffalo bulls produced from 34 sires and 92 dams during 10 years from 2013 to 2022 in two herds of the International Livestock Management Training Center at Sakha (IMTC) and Mahalet Mousa (MM), Kafr El-Sheikh Governorate. Data on body weight at birth and weaning were collected from 8229 buffalo calves, progeny of 277 sires

and 2175 dams for a period of 22 years from 2003 to 2024 in six experimental herds of NG, NK, EN, ES, EG and S. All the herds are belonging to the Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agricultural and Land Reclamation (MALR), Egypt.

For molecular analyses in this study, blood samples from 286 animals (200 female and 86 male) in three herds of NG, NK, and EG were randomly collected from buffalo animals for genotyping using PCR-RFLP technique. The candidate genes of *GH*, *PRL* and *FSHR* were investigated in terms of the association of these genes with lactation, reproduction, semen and growth traits.

5.1 Quantitative genetic analyses for lactation traits

Heritability values estimated by repeatability single-trait animal model for lactation traits were mostly moderate, ranging from 0.05 to 0.40 for TDMY, 0.05 to 0.45 for TDFY, 0.06 to 0.44 for TDPY and 0.03 to 0.39 for TDSCS, while those values estimated by RRM for lactation traits were mostly low at the beginning of lactation, increased gradually to reach the highest value then decreased gradually to reach the lowest value towards the end of lactation. The heritabilities estimated by RRM ranged from 0.04 to 0.25 for TDMY, 0.05 to 0.18 for TDFY, 0.03 to 0.23 for TDPY and 0.07 to 0.57 for TDSCS. The ranges in PBVs for lactation traits were moderate or high, being -2.01 to 3.4 kg for TDMY, -358 to 521 g for TDFY, -53 to 95 g for TDPY and -0.183 to 0.313 $\log^{10}$ for TDSCS. The plotted genetic trends for lactation traits were increased favorably from -4.63 to 1.61 kg for TDMY, -5.0 to 495 g for TDFY and -26 to 280 g for TDPY, along with favorable decrease of 1.37 to 1.19 $\log^{10}$ in the genetic trend of TDSCS over time of lactation. On the contrary, the phenotypic trends of lactation traits were decreased unfavorably from 7.49 *kg* to be 5.69 *kg* for TDMY, 510 *g* to be 360 *g* for TDFY and 284 *g* to be 223 *g* for TDPY with unfavorable increase from 1.62 to be 2.43 $\log^{10}$ for TDSCS.

5.2 Quantitative genetic analyses for reproduction traits

Heritability estimated by single-trait animal model for reproduction traits were low, being 0.10 for AFC, 0.02 for DO and 0.02 for CI. The ranges in PBVs were moderate or high, being -8.24 to 10.84 *mo* for AFC, -124.7 to 123.9 *d* for DO and -141.8 to 132.5 *d* for CI. The genetic trends were favorably decreased from 0.24 *mo* to be -0.14 *mo* for AFC, 5.5 *d* to be 2.9 *d* for DO and 6.9 *d* to be 3.6 *d* for CI. Wide ranges in values of the phenotypic trends of reproduction traits were observed, in terms of 36.6 *mo* to be 36.5 *mo* for AFC, 127 *d* to be 71 *d* for DO and 416 *d* to be 354 *d* for CI.

5.3 Quantitative genetic analyses for semen traits

Heritability estimated by single-trait animal model for semen traits were moderate, being 0.17, 0.28, 0.27, 0.27 and 0.23 for ejaculate volume (EV), motility of sperms (MS), live sperms (LS), abnormal sperms (AS) and sperms concentration (SC), respectively. The ranges in PBVs were moderate or high, being -0.63 to 0.42 ml for EV, -27.3 to 85.0 % for MS, -27.3 to 81.7 % for LS, -3.7 to 24.8 % for AS and -1.2 to 2.5×10^9 sperm per ml for SC. The genetic trends for semen traits were increased favorably over time from 1.99 to 2.3 ml for EV, 36.8 to 47.8 % for MS, 35.6 to 47.8 % for LS, 2.3 to 5.9% for AS and 0.39 to 1.24×10^9 sperm per ml for SC. The phenotypic trends for EV, MS and LS were decreased from 4.1 to 3.1 ml for EV, 68.2 to 57.1 % for MS and 67.4 to 56.2 for LS, while the trends were increased from 3.1 to 8.1% for AS and from 0.6 to 1.3×10^9 sperm per ml for SC.

5.4 Quantitative genetic analyses for growth traits

Heritability values estimated by animal model for body weight at birth (BW), weaning weight (WW) and daily gain from birth to weaning (DG) were mostly moderate or high, being 0.26 for BW, 0.50 for WW and 0.55 for DG. The PBVs ranging from -4.2 to 3.5 kg for BW, -42.4 to 44.2 kg for WW and -0.44 to 0.52 kg for DG. The genetic trends for body weights and gains increased slightly favorably from 1.6 to 1.8 *kg* for BW, -

0.519 to 1.57 *kg* for WW and -24 to 18 *g* for DG. The ranges in values of the phenotypic trends for body weights and gains decreased slightly from 36.6 to 32.9 *kg* for BW, 94.55 to 94.15 *kg* for WW and 628 to 582 *g* for DG.

5.5 Polymorphic characterization of *PRL, DGAT1, FSHR* and *GH* gene in different herds:

The molecular weights of *PRL* gene, the amplified DNA fragment of 678 *bp* was digested using the *Xba*I restriction enzyme, where one band in AA genotype (678 bp) and two bands in GG genotype (678 and 447 bps) were detected. The genotypic frequency of AA genotype of *PRL* gene was high (0.851) and the frequency of GG genotype was low (0.149). Also, the allelic frequency recorded for A allele was much higher than that recorded for G allele (0.851 vs 0.149). In comparing NG herd with NK herd, the frequency of AA and GG genotypes of *PRL* gene were nearly similar (0.900 vs 0.845 for AA genotype; 0.100 vs 0.155 for GG genotype). The effective numbers of alleles (*Ne*) as an index of genetic diversity revealed that the difference in *Ne* between NG and NK herds was significant (1.220 *vs* 1.355, $P<0.01$). The values of polymorphic information content (*PIC*) were low in NG herd (0.157), moderate value of 0.223 in NK herd and moderate value of 0.211 in both herds. Chi-square values ($\chi 2$) for genotypes of *PRL* gene were highly significant in NG and NK herd, indicnting that both herds are in of *HWE* for this gene. The expected heterozygosity values (H_E) for *PRL* gene were moderate with expected values to be 0.180 in NG herd, 0.262 in NK and 0.253 in both herds.

The genotypic frequency of genotype CC for *DGAT1* gene was 100% with frequency of 1.0 for allele C and 0.0 for allele T. A PCR amplified DNA with fragment length of 411 *bp* was digested using *AluI* restriction enzyme and one monomorphic CC genotype was detected for *DGAT1* gene, getting three bands with fragment length of 176, 167 and 68 bps.

For *FSHR* gene, the amplified DNA fragment of 306 *bp* was digested using *AluI* restriction enzyme and three genotypes (GG, GC and CC) were obtained. The PCR products were one band in GG genotype (306 bp), two bands in CC genotype (243 and 63 bp*s*) and three bands in GC genotype (306, 243 and 63 bp*s*). The genotypic frequencies for genotypes of *FSHR* gene for buffalo cows and bulls were 0.41 for GC genotype, 0.21 for GG genotype and 0.38 for CC genotype, *i.e*. allelic frequency for C allele (0.592) was higher than that for G allele (0.408). Also, the frequency for GG, GC and CC genotypes of *FSHR* gene were 0.14, 0.51 and 0.35 for buffalo cows and 0.28, 0.28 and 0.44 for buffalo bulls. The frequency of GG, GC and CC genotypes of *FSHR* gene in NG, NK and EG herds were widely differed (0.212 in NG herd, 0.113 in NK herd and 0.095 in EG herd for GG genotype; 0.515 in NG herd, 0.500 in NK herd and 0.534 in EG herd for GC genotype; 0.272 in NG herd, 0.386 in NK herd and 0.381 in EG herd for CC genotype). The frequencies for C allele were higher than those for G allele where the frequencies were 0.530, 0.636 and 0.643 for C allele vs 0.470, 0.364 and 0.357 for G allele in NG, NK and EG herds, respectively. The differences in *Ne* among NG, NK and EG herds for *FSHR* gene were significant ($P<0.01$) where N_e were 1.993, 1.862 and 1.849 in NG, NK and EG herds, respectively. The current *PIC* values were moderate and varied from 0.531 in NG herd to 0.653 and 0.662 in NK and EG herds, respectively. The values of expected heterozygosity (H_E) for *FSHR* gene were high, being 0.498 in NG herd, 0.463 in NK herd, 0.459 in EG herd, 0.479 in buffalo cows and 0.488 in buffalo bulls, while the observed heterozygosities (H_O) were 0.515 in NG herd, 0.500 in NK herd, 0.524 in EG herd, 0.510 in buffalo cows and 0.282 in buffalo bulls.

For *GH* gene, the PCR amplified DNA fragment with length of 211 *bp* was digested using *AluI* restriction enzyme and two genotypes of CC and TC were obtained. The PCR product yielded banding pattern corresponding to two bands of 211 and 159 bp*s* for CC genotype and three bands of 211, 159 and 52 bp*s* for TC genotype. The frequencies of CC genotype for *GH* gene were 0.608 in NG herd, 0.505 in NK herd and 0.500 in EG herd, while

the frequencies of TC genotype were 0.392 in NG herd, 0.495 in NK herd and 0.500 in EG herd. Across all herds, the frequencies of CC genotype were 0.68 for females, 0.30 for males and 0.52 for both sexes, while the frequencies of TC genotype were 0.32 for females, 0.70 for males and 0.48 for both sexes. The frequencies recorded for C allele (0.804 in NG herd, 0.753 in NK herd and 0.750 in EG herd) were higher than those recorded for T allele (0.196 in NG herd, 0.247 in NK herd and 0.250 in EG herd). The difference in allelic numbers among the three herds were significant ($P<0.01$). Across all herds, the highest *Ne* was obtained for buffalo bulls (1.839), while the lowest allelic number was obtained for buffalo cows (1.368). The Chi-square values for genotypes of *GH* gene were not significant in buffalo cows and buffalo bulls, indicating that these populations were in *Hardy-Weinberg equilibrium* (*HWE*). Across the herds, the values of heterozygosity for *GH* gene were moderate to high and ranged from 0.320 to 0.704 for H_O and 0.269 to 0.456 for H_E.

5.6 Molecular associations between genotypes of *PRL, FSHR* or *GH* gene and lactation traits

The differences in GLSM estimated by PEST software for lactation traits between AA and GG genotypes of *PRL* gene in NG and NK herds were mostly significantly in favour of AA genotype ($p<0.01$). In both NG and NK herds, GLSM recorded for AA genotype were high being 6.0 kg for TDMY, 390 g for TDFY, 290 g for TDPY and 2.47 $\log^{10}$ for TDSCS compared with 5.3 kg, 340 g, 220 g and 2.50 $\log^{10}$ for GG genotype, respectively. In NG herd, GLSM for lactation traits were significantly in favour of AA genotype of *PRL* gene relative to GG genotype in terms of 5.9 *vs* 5.5 kg for TDMY, 360 *vs* 310 g for TDFY, 260 *vs* 220 g for TDPY and 2.38 *vs* 2.52 $\log^{10}$ for TDSCS, while the respective GLSM in NK herd were 5.97 *vs* 5.43 kg, 390 *vs* 350 g, 290 *vs* 230 g and 2.41 *vs* 2.49 $\log^{10}$.

The differences in GLSM for most lactation traits among GG, GC and CC genotypes of *FSHR* gene in NG and NK herds were significantly in favour of CC genotype ($P<0.01$). GLSM for lactation traits in NG herd

were significantly in favour of CC genotype of *FSHR* gene relative to CG and GG genotypes in terms of 6.8 kg *vs* 5.7 and 6.0 kg for TDMY, 480 g *vs* 380 and 410 g for TDFY, 280 g *vs* 220 and 250 g for TDPY and 2.41 $\log^{10}$ *vs* 2.43 and 2.49 log10 for TDSCS, while the corresponding GLSM in NK herd were 6.8 kg *vs* 5.4 and 5.5 kg, 390 g *vs* 340 and 320 g, 290 g *vs* 250 and 230 g and 2.41 $\log^{10}$ *vs* 2.45 and 2.49 $\log^{10}$.

Two genotypes of TC and CC for *GH* gene in each NG and NK separate herds were significantly in favour of TC genotype for lactation traits (*P<0.01*). The GLSM for TC genotype of *GH* gene were significantly higher in lactation traits than that of CC genotype in terms of 6.3 *vs* 5.8 kg for TDMY, 480 *vs* 380 g for TDFY and 290 *vs* 230 g for TDPY in NG herd and 6.3 *vs* 5.6 kg, 390 *vs* 350 g and 290 *vs* 230 g in NK herd. Also, GLSM of TDSCS were in favour of TC genotype relative to CC genotype (2.41 $\log^{10}$ in NG herd and 2.45 $\log^{10}$ in NK herd).

5.7 Molecular associations between genotypes of *PRL, FSHR* or *GH* gene and reproduction traits

For most reproduction traits, the GLSM for AA and GG genotypes of *PRL* gene showed that these genotypes were significantly molecularly associated with AFC, DO and CI. Also, the differences in GLSM between AA and GG genotypes of *PRL* gene for AFC, DO and CI were most significantly in favour of GG genotype relative to AA genotype in NG, NK and EG herds (*P<0.01*) where, GLSM for GG genotype ranging from 33.8 to 41 *mo* for AFC, 142 to 170 *d* for DO and 435 to 469 *d* for CI. In NG herd, GLSM for GG genotype of *PRL* gene were significantly favourable for reproduction traits compared to AA genotype in terms of 41.0 *vs* 43.0 *mo* for AFC, 142 *vs* 174 *d* for DO and 345 *vs* 476 *d* for CI, while the corresponding GLSM in NK were 33.8 *vs* 35.1 *mo*, 143 *vs* 158 *d* and 449 *vs* 459 *d*.

The differences in GLSM for reproduction traits among GG, GC and CC genotypes of *FSHR* gene were significantly in favour of CC genotype (*P<0.01*). The GLSM recorded for CC genotype of *FSHR* gene were significantly favourable lower than GLSM for GC and GG genotypes, being

37.9 *mo vs* 39.7 and 42.5 *mo* for AFC, 83 *d vs* 91 and 102 *d* for DO and 387 *d vs* 397 and 419 *d* for CI in NG herds, comparable with 32.0 *mo vs* 34.3 and 35.6 *mo*, 83 *d vs* 91 and 102 *d* and 384 *d vs* 408 and 398 *d* in NK herd, respectively. Also, favourable trends were observed in EG herd where GLSM were 35.0 *mo vs* 37.3 and 36.5 *mo* for AFC, 103 *d vs* 109 and 118 *d* for DO and 366 *d vs* 396 and 410 *d* for CI.

Two genotypes of *GH* gene (TC and CC) were detected for reproduction traits in NG, NK and EG herds and the differences between both genotypes were significantly in favour of TC genotype. The GLSM recorded for TC genotype in NG, NK and EG herds were 37.8, 33.7 and 35.4 *mo* for AFC, 93, 94 and 105 *d* for DO and 383, 379 and 395 *d* for CI, comparable with the corresponding GLSM of 41.4, 35.2 and 37.5 *mo* for AFC, 115, 100 and 121 *d* for DO and 407, 393 and 406 *d* for CI (*P<0.01*).

5.8 Molecular associations between genotypes of *FSHR* or *GH* gene and semen traits

The differences in GLSM for semen traits among GG, GC and CC genotypes of *FSHR* gene were significantly in favour of GG genotype (*P<0.01*). These significant differences in GLSM were 2.9 ml vs 2.6 and 2.5 ml for EV trait, 64.1 % vs 59.3 and 63.2 % for MS trait, 62.8 % vs 57.8 and 61.9 % for LS trait, 9.1% vs 9.9 and 9.4% for AS trait, 1.59×10^9 sperm per ml vs 1.36 and 1.50×10^9 sperm per ml for SC trait.

For *GH* gene, the associations were significantly in favour of CC genotype relative to TC genotype (*P<0.01*) where GLSM for semen traits were 2.9 vs 2.5 *ml* for EV trait, 64.1 vs 60.9 % for MS trait, 62.1 vs 60.0 % for LS trait, 8.9 vs 9.6 % for AS trait, 1.60×10^9 vs 1.40×10^9 sperm per ml for SC trait.

5.9 Molecular associations between genotypes of *GH, PRL* or *FSHR* gene and growth traits

The associations were significantly (*P<0.01*) in favour of TC genotype relative to CC genotype in NG, NK and EG herds. Across all herds, GLSM for TC genotype had significantly heavier body weights and gains than CC

genotype (36.8 *vs* 33.9 *kg* for BW, 96.3 *vs* 91.8 *kg* for WW and 600 *vs* 540 *g* for DG). For each experimental herd, GLSM of birth weight (BW), weaning weight (WW) and daily gain (DG) of TC genotype were favorably heavier relative to CC genotype (36.9 *kg*, 94.8 *kg* and 590 *g vs* 34.4 *kg*, 91.2 *kg* and 560 *g* in NG herd; 38.0 *kg*, 95.9 *kg* and 580 *g vs* 36.5 *kg*, 90.9 *kg* and 530 *g* in NK herd; 39.0 *kg*, 104.6 *kg* and 660 *g vs* 33.0 *kg*, 91.5 *kg* and 530 *g* in EG herd).

Two dimorphic genotypes of AA and GG for *PRL* gene were identified in each herd (NG, NK and EG) where GLSM for AA genotype across herds was significantly heavier in BW and DG than GG genotype (38.6 *vs* 36.1 *kg* for BW, 93.7 *vs* 90.8 *kg* for WW and 594 *vs* 568 *g* for DG). Similarly, the GLSM in each separate herd for BW, WW and DG of AA genotype were favorably higher in weights and gains relative to GG genotype (43.9 *kg*, 95.9 *kg* and 594 *g vs* 33.9 *kg*, 92.6 *kg* and 472 *g* in NG herd; 36.4 *kg*, 95.0 *kg* and 605 *g vs* 34.0 *kg*, 90.8 *kg* and 542 *g* in NK herd; 43.7 *kg*, 99.9 *kg* and 623 *g vs* 34.8 *kg*, 77.6 *kg* and 367 *g* in EG herd).

Three genotypes of GG, GC and CC for *FSHR* gene were identified (Polymorphic). The differences in GLSM among these genotypes across herds for BW, WW and DG were significantly in favour of GG genotype relative to GC and CC genotypes ($P<0.01$), where GLSM for GG genotype were significantly heavier in weight and gain than GC and CC genotypes (38.8 *kg vs* 35.4 and 36.8 *kg* for BW; 97.9 *kg vs* 92.8 and 92.6 *kg* for WW; 603 *g vs* 558 and 452 *g* for DG). In each herd, GLSM of GG genotype for BW, WW and DG were favorably heavier in weighs and gains relative to GC and CC genotype (37.7 *kg*, 102.9 *kg* and 662 *g* for GG genotype *vs* 32.9 *kg*, 89.1 *kg* and 550 *g* for GC genotype and 36.3 *kg*, 88.8 *kg* and 524 *g* for CC genotype in NG herd; 38.1 *kg*, 95.3 *kg* and 577 *g* for GG genotype *vs* 36.5 *kg*, 92.8 *kg* and 552 *g* for GC genotype and 36.5 *kg*, 92.5 *kg* and 537 *g* for CC genotype in NK herd). Oppositely, GLSM for BW, WW and DG of CC genotype in EG herd were significantly heavier than GG and GC genotypes (39.4 *kg*, 106.2 *kg* and 636 *g* for CC genotype *vs* 33.3 *kg*, 93.3 *kg* and 574 *g* for GC genotype and 35.5 *kg*, 92.5 *kg* and 548 *g* for GG genotype).

6. CONCLUSIONS

6.1 Impacts to be considered for lactation and reproduction traits in buffalo

- The test-day (TD) lactation traits during the first three to five months of lactation could be adopted as an early selection criterion to increase milk yield and components in buffalo.
- The favorable genetic trends for lactation and reproduction traits obtained in the present study could be dedicated to the fact that it is necessary to improve the management and feeding scheme and to use accurate estimates of predicted breeding values in the genetic improvement programs of Egyptian buffalo.
- RRM parameters and PBV and genetic and phenotypic trends estimated in the present study could be safely used in the genetic improvement programs in Egyptian buffalo of APRI herds. However, subsequent work is needed to evaluate the applicability of such analyses under the conditions of some private farms scattered in the Egyptian countryside.
- The significant molecular associations detected between AA genotype of *PRL* gene, CC genotype of *FSHR* gene and TC genotype of *GH* gene and lactation and reproduction traits may be advantageous for marker-assisted selection programs aiming to improve lactation traits (milk, fat, protein and somatic cell score) and reproduction performance (age at first calving, days open and calving interval) in Egyptian buffalo.

6.2 Impacts to be considered for growth and semen traits in buffalo

- Birth or weaning weight could be adopted as an early selection criterion to improve growth performance in Egyptian buffalo.
- Semen traits could be adopted as selection criteria to improve reproductive performance in Egyptian buffalo bulls. Improving management and feeding schemes and using accurate estimates of

predicted breeding values in the genetic improvement programs in Egyptian buffalo, should improve semen traits of bulls efficiently.

- Based on the significant molecular associations detected between TC genotype of *GH* gene, AA genotype of *PRL* gene and GG genotype of *FSHR* gene and body weights could be used as advantageous marker-assisted tools in selection programs, aiming to improve body weights and semen traits in Egyptian buffalo.

6.3 Recommendations

- Practically, the Egyptian buffalo geneticists can use AA genotype of *PRL* gene and TC genotype of *GH* gene in marker-assisted selection to improve lactation and growth traits; GG genotype of *PRL* gene to improve reproductive performance of buffalo cow; GG genotype of *FSHR* to improve growth performance and reproduction traits of buffalo bulls; CC genotype of *FSHR* gene to enhance milk and fertility traits of buffalo cows and CC genotype of *GH* gene to improve fertility of buffalo bulls and cows.

7. REFERENCES

Abd El Fattah, E.M., Behour, T.S., Ashour, A.F. and Amin, A.M.S. 2023. Association analysis of prolactin and prolactin receptor genes with selected productive and reproductive traits in Egyptian buffalo. *Animal Biotechnology*, 34 (4), 1397-1405. https://doi.org/10.1080/10495398.2022.2028160.

Abdel-Salam, S.A.M., Abou-Bakr, S., Ibrahim, M.A.M., Sadek, R.R. and Abdel-Aziz, A.S. 2009. Estimation of breeding values of total milk yield of Egyptian buffalo under different production systems. *"ICAR Technical Series, issue 13, International Committee for Animal Recording (ICAR*), 167-172.

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

Abdo, S.E., Abu El-Magd, M., ElNahas, A.F. and Mansour, A.E.K. 2014. Leptin gene *SNPs* and their effect on milk traits in Egyptian buffalo (Bubalus bubalis*). Kafrelsheikh Veterinary Medical Journal*, 12(1), 97-110. https://doi.org/10.21608/kvmj.2014.110129.

Abo-Al-Ela, H.G., El-Magd, M.A., El-Nahas, A.F. and Mansour, A.A. 2014. Association of a novel SNP in exon 10 of the IGF2 gene with growth traits in Egyptian water buffalo (Bubalus bubalis). *Tropical Animal Health and Production*, 46, 947-952. https://doi.org/10.1007/s11250-014-0588-3.

Abo-Gamil, Z.H.S., El-Bayomi, K.M., Abo-Salem, M.E.S., Ramadan, S. and Darwish, S.A. 2017. Estimation of gentic and phenotypic parameters for some productive and reproductive traits in Egyptian buffoloes. *Benha Journal of Applied Sciences*, 2(3), 53-59. https://doi.org/10.21608/bjas.2017.164545.

Afifi, E.A., Khalil, M.H., Bedeir, L.H. and Zeidan, S.M. 1992. Genetic analysis of reproductive traits in Egyptian buffaloes. *Egyptian Journal of Animal Production*, 29(2), 139-154.

Afshar, K.P., Javanmard, A., Asadzadeh, N., Sadeghipanah, H., Masomi, H. and Sabrivand, A. 2011. Association between *GH* encoding gene polymorphism and semen characteristics in Iranian Holstein bulls. *African Journal of Biotechnology*, 10(6), 882-886. https://doi.org/10.5897/AJB10.1373.

Agudelo-Gómez, D., Pineda-Sierra, S. and Cerón-Muñoz, M.F. 2015. Genetic evaluation of dual-purpose buffaloes (Bubalus bubalis) in Colombia using principal component analysis. *PloS One*, 10(7), e0132811. https://doi.org/10.1371/journal.pone.0132811.

Ahmad, M. 2007. Estimated breeding values and genetic trend for milk yield in Nili Ravi buffaloes. *Italian Journal of Animal Science*, 6(Sup2), 393-396. https://doi.org/10.4081/ijas.2007.s2.393.

Ahmad, M., Parveen, A., Ghaffar, A. and Aziz, M.M. 2008. Estimated breeding values and genetic trend for 305-day milk yield in buffalo herd at LES Chak Katora. *Pakistan Journal of Agricultural Sciences*, 45(2), 212-214.

Ahmad, A.I., El-Komy, M.S., Awad, S.H.S and Khattab, A.S. 2017. Phenotypic and genotypic trends for some economic Traits in Egyptian buffaloes. *Journal of Animal and Poultry Production*, Mansoura University, 8(6), 129-133. https://doi.org/10.21608/jappmu.2017.45797.

Ahmadzadeh, M., Rashidi, F., Amirpour Najafabadi, H., Jaferian, A. and Eghbalsaied, S. 2019. Effects of genetic polymorphism in Pit1, GH, GHR and KCN3 on milk yield and body weight of Khuzestan (Iran) water buffaloes. *Revista Colombiana de Ciencias Pecuarias*, 32(2), 107-116. https://doi.org/10.17533/udea.rccp.v32n2a04.

Ali, M., Ahmed, S., Ali, M., Ahmad, N., Tariq, M.N., Usman, M. and Sardar, A.A. 2023. Effects of saccharomyces cerevisiae live cells and

culture on growth and productive performance in lactating Nili-Ravi buffaloes. *Tropical Animal Health and Production*, 55(4), 283.

Al-Kal, A., Jiang, Q.Y., Qin, G.S., Pan, B., Chen, B.J. and Jiang, H.S., 2018. Associations between prolactin receptor (PRLR) polymorphisms and milk production traits in dairy buffalo. *Canadian Journal of Animal Science*, 99(2), 254-259. https://doi.org/10.1139/cjas-2018-0050.

Al-Shawa, Z., El-Zarei, M., Ghazy, A., Amin, A, Ayoub, M., Merdan, S. and Mokhtar, S. 2019.. Genetic profile of κ-casein gene based on RFLP technique in association with milk traits in Egyptian buffaloes. *Journal of Animal, Poultry & Fish Production*, 8(1), 9-20.

Amimo, J.O., Mosi, R.O., Wakhungu, J.W., Muasya, T.K. and Inyangala, B.O. 2006. Phenotypic and genetic parameters of reproductive traits for Ayrshire cattle on large-scale farms in Kenya. . *Livestock Research for Rural Development*, 18(10).

Amimo, J.O., Wakhungu, J.W., Inyangala, B.O., and Mosi, R.O. 2007. The effects of non-genetic factors and estimation of genetic and phenotypic parameters and trends for milk yield in Ayrshire cattle in Kenya. *Livestock Research for Rural Development*, 19(1), 1-9. http://www.cipav.org.co/lrrd19/1/amim19013.htm.

Amin, A.M.S., Khalil, M.H., Mourad, K.A., Afifi, E.A. and Ibrahim, M.K. 2015. Genetic and phenotypic trends for test day milk, fat and protin yields applying random regression model in Egyptian buffaloes. *Egyptian Journal of Animal Production*, 52(4), 19-29.

Amin, A.M., Abo-Ismail, M.K. and Salem, M.M. 2021. Genetic parameters and genetic trends for reproductive traits in Egyptian buffalo. *Animal Reproduction Science*, 231, 106800. https://doi.org/10.1016/j.anireprosci.2021.106800.

Amin, A.M., Salem, M.M., Ashour, A.F. and El Nagar, A.G. 2024. Principal component analysis of phenotypic and breeding value data for semen traits in Egyptian buffalo bulls. *Tropical Animal Health and Production*, 56(4), 135. https://doi.org/10.1007/s11250-024-03975-3.

Andreas, E., Sumantri, C., Nuraini, H., Farajallah, A. and Anggraeni, A. 2010. Identification of GH AluI and GHR AluI genes polymorphisms in Indonesian buffalo. *Journal of the Indonesian Tropical Animal Agriculture*, 35(4), 215-221. https://doi.org/10.14710/jitaa.35.4.215-221.

Anggraeni, A., Thalib, C. and Saputra, F. 2023. GH, GHRH, and PIT genes polymorphisms of local Swamp buffalo in pandeglang district, banten province. *Advances in Biological Sciences Research*, 28, 50–59. https://doi.org/10.1051/bioconf/20213302005.

Araújo, C.V.D., Torres, R.D.A., Costa, C.N., Torres Filho, R.D.A., Araújo, S.I., Lopes, P.S., Regazzi, A.J., Pereira, C.S. and Sarmento, J.L.R. 2006. Uso de modelos de regressão aleatória para descrever a variação genética da produção de leite na raça Holandesa. *Revista Brasileira de Zootecnia*, 35, 975-981. https://doi.org/10.1590/S1516-35982006000400006.

Araújo, R.O.D., Rorato, P.R.N., Weber, T., Everling, D.M., Lopes, J.S. and Dornelles, M.D.A. 2010. Genetic parameters and phenotypic and genetic trends for weight at weaning and visual scores during this phase estimated for Angus-Nellore crossbred young bulls. *Revista Brasileira de Zootecnia*, 39, 2398-2408. https://doi.org/10.1590/S1516-35982010001100012.

Araújo, D.N., de Camargo, G.M.F., Fonseca, P.D.D.S., Cardoso, D.F., Hurtado-Lugo, N.A., Aspilcueta-Borquis, R.R. and Tonhati, H. 2015. Polymorphisms in oxytocin and α1a adrenergic receptor genes and their effects on production traits in dairy buffaloes. *Animal Biotechnology*, 26(3), 165-168. https://doi.org/10.1080/10495398.2013.877918.

Arslan, K., Akyüz, B. and Agaoglu, O.K. 2015. Investigation of STAT5A, FSHR, and LHR gene polymorphisms in Turkish indigenous cattle breeds (East Anatolian Red, South Anatolian Red, Turkish Grey, Anatolian Black, and Zavot). *Russian Journal of Genetics*, 51, 1088-1095. https://doi.org/10.1134/S1022795415110022.

Ashmawy, A. and El-Bramony, M.M. 2017. Genetic association for some growth and reproductive traits in primiparous buffalo females. *International Journal of Genetics*, 7, 25-30. https://doi.org/10.5829/idosi.ijg.2017.25.30.

Aspilcueta-Borquis, R., Tanaka, A., Albuquerque, L., Sesana, R., Seno, L. and Tonhati, H. 2007. Genetic parameters estimates for milk, fat and protein yield analyzed by test day models for Murrah buffaloes in Brazil. Italian *Journal of Animal Science*, 6(Sup2), 368-371. https://doi.org/10.4081/ijas.2007.s2.368.

Aspilcueta-Borquis, R.R., Sesana, R.C., Berrocal, M.H.M., Seno, L.D.O., Bignardi, A.B., El Faro, L., Albuquerque, L.G.D., Camargo, G.M.F.D. and Tonhati, H. 2010. Genetic parameters for milk, fat and protein yields in Murrah buffaloes (Bubalus bubalis Artiodactyla, Bovidae). *Genetics and Molecular Biology*, 33, 71-77. https://doi.org/10.1590/S1415-47572010005000005.

Aspilcueta-Borquis, R.R., Neto, F.R.A., Baldi, F., Santos, D.J., Albuquerque, L.G. and Tonhati, H. 2012. Genetic parameters for test-day yield of milk, fat and protein in buffaloes estimated by random regression models. *Journal of Dairy Research*, 79(3), 272-279. https://doi.org/10.1017/S0022029912000143.

Aspilcueta-Borquis, R.R., Neto, F.A., Santos, D.J.A., Hurtado-Lugo, N.A., Silva, J.A.V. and Tonhati, H. 2015. Multiple-trait genomic evaluation for milk yield and milk quality traits using genomic and phenotypic data in buffalo in Brazil. *Genetics and Molecular Research*, 14(4), 18009-18017. https://doi.org/10.3168/jds.2009-2621.

Ayuk, J. and Sheppard, M.C. 2006. Growth hormone and its disorders. *Postgraduate Medical Journal*, 82(963), 24-30. https://doi.org/10.1136/pgmj.2005.036087.

Aziz, M.A., Schoeman, S.J., Jordaan, G.F., El-Chafie, O.M. and Mahdy, A.T. 2001. Genetic and phenotypic variation of some reproductive traits

in Egyptian buffalo. *South African Journal of Animal Science*, 31(3), 195-199. https://doi.org/10.4314/sajas.v31i3.3802.

Babera, S.C., Uy, M.R.D., Tubalinal, G.A.S.P., Mamuad, F.V. and Mingala, C.N. 2022. Molecular characterization and polymorphism of inhibin (INHβA) gene in Water Buffalo (Bubalus bubalis) bulls. *Philippine Journal of Science*, 151, 275-280. https://doi.org/10.56899/151.01.20.

Barbas, J.P. and Mascarenhas, R.D. 2009. Cryopreservation of domestic animal sperm cells. *Cell and Tissue Banking*, 10, 49-62. https://doi.org/10.1007/s10561-008-9081-4.

Barros, C.D.C., Aspilcueta-Borquis, R.R., Fraga, A.B. and Tonhati, H. 2016. Genetic parameter estimates for production and reproduction traits in dairy buffaloes. *Revista Caatinga*, 29(01), 216-221. https://doi.org/10.1590/1983-21252016v29n125rc.

Bashir, M.K., Khan, M.S., Lateef, M. and Akhtar, P. 2009. Genetic and phenotypic aspects of age at first calving of Nili-Ravi buffaloes in Pakistan. *Pakistan Journal of Zoology Society*, 9, 757-61.

Bhave, K., Koilpillai, T.P.J., Ragothaman, V., Sontakke, S., Joshi, G. and Ducrocq, V. 2020. Semen production and semen quality of indigenous buffalo breeds under hot semiarid climatic conditions in India. *Tropical Animal Health and Production*, 52, 2529-2539. https://doi.org/10.1007/s11250-020-02284-9.

Bignardi, A.B., El Faro, L., Cardoso, V.L., Machado, P.F. and de Albuquerque, L.G., 2009. Random regression models to estimate test-day milk yield genetic parameters Holstein cows in Southeastern Brazil. Livestock Science, 123(1), 1-7. https://doi.org/10.1016/j.livsci.2008.09.021.

Butler, M.L., Hartman, A.R., Bormann, J.M., Weaber, R.L., Grieger, D.M. and Rolf, M.M. 2021. Genetic parameter estimation for beef bull semen attributes. *Journal of Animal Science*, 99(2), 1-11. https://doi.org/10.1093/jas/skab013.

Caivio-Nasner, S.L., López-Herrera, A., González-Herrera, L.G. and Flórez, J.C.R. 2021. Genetic parameters and trends for reproductive traits in Blanco Orejinegro cattle from Colombia. Semina: *Ciências Agrárias*, 42(4), 2523-2538. https://doi.org/10.5433/1679-0359.2021v42n4p2523.

Carvalho Filho, I., Marques, D.B.D., de Campos, C.F., Guimarães, J.D., Guimarães, S.E.F., Lopes, P.S., Silva, F.F.E. and Veroneze, R. 2020. Genetic parameters for fertility traits in Nellore bulls. *Reproduction in Domestic Animals*, 55(1), 38-43. https://doi.org/10.1111/rda.13578.

Cassiano, L.A.P., Mariante, A.D.S., McManus, C., Marques, J.R.F. and Costa, N.A.D. 2004. Genetic parameters of production and reproduction traits of buffaloes in the Brazilian Amazon. *Pesquisa Agropecuária Brasileira*, 39, 451-457. https://doi.org/10.1590/S0100-204X2004000500007.

Catillo, G., Moioli, B. and Napolitano, F. 2001. Estimation of genetic parameters of some productive and reproductive traits in Italian buffalo. Genetic evaluation with BLUP-Animal Model. *Asian-Australasian Journal of Animal Sciences*, 14(6), 747-753. https://doi.org/10.5713/ajas.2001.747.

Chakraborty, D. and Dhaka, S.S. 2012. Estimation of genetic and phenotypic trends from production efficiency traits in Murrah buffaloes. *Indian Journal of Animal Research*, 46(4), 410-412.

Chakraborty, D., Dhaka, S., Pander, B.L. and Yadav, A.S. 2010. Genetic studies on production efficiency traits in Murrah buffaloes. *The Indian Journal of Animal Sciences*, 80(9), 898-901.

Chandra, S., Das, D.N., Kannegundla, U., Reen, J.K., Ramesha, K.P., Nath, S. and Kataktalware, M.A. 2020. Polymorphism in inhibin alpha gene and its association with semen quality traits in Murrah bulls. *Indian Journal of Animal Research*, 54(4), 399-404. https://doi.org/10.18805/ijar.B-3798.

Chaudhary, C.S., Tripathi, V.N. and Gupta, A.K. 1995. Genetic, phenotypic and environmental trend for reproduction traits in Kankrej cattle. *The Indian Journal of Animal Sciences*, 65(1), 106-107.

Chu, S. and Majumdar, A. 2012. Opportunities and challenges for a sustainable energy future. *Nature*, 488(7411), 294-303. https://doi.org/10.1007/s11033-011-1147-x.

Cobuci, J.A., Costa, C.N., Braccini Neto, J. and Freitas, A.F.D. 2011. Genetic parameters for milk production by using random regression models with different alternatives of fixed regression modeling. *Revista Brasileira de Zootecnia*, 40, 557-567. https://doi.org/10.1590/S1516-35982011000300013.

Cosenza, G., Macciotta, N.P., Nudda, A., Coletta, A., Ramunno, L. and Pauciullo, A. 2017. A novel polymorphism in the oxytocin receptor encoding gene (OXTR) affects milk fatty acid composition in Italian Mediterranean river buffalo. *Journal of Dairy Research*, 84(2), 170-180. https://doi.org/10.1017/S0022029917000127.

Cosenza, G., Gallo, D., Auzino, B., Gaspa, G. and Pauciullo, A. 2021. Complete CSN1S2 characterization, novel allele identification and association with milk fatty acid composition in river buffalo. *Frontiers in Genetics*, 11, 622494. https://doi.org/10.3389/fgene.2020.622494.

Costa, M., Goldberger, A.L. and Peng, C.K. 2005. Multiscale entropy analysis of biological signals. *Physical Review E—Statistical, Nonlinear, and Soft Matter Physics*, 71(2), 021906. https://doi.org/10.1103/PhysRevE.71.021906.

Costa, A., Negrini, R., De Marchi, M., Campanile, G. and Neglia, G. 2020. Phenotypic characterization of milk yield and quality traits in a large population of water buffaloes. *Animals*, 10(2), 327. https://doi.org/10.3390/ani10020327.

Darwish, S.F., Tarek, S.H. and Badr, M.R. 2016. Association between genotypes of growth hormone gene and semen quality traits in cow

bulls and buffalo bulls. *Alexandria Journal of Veterinary Sciences*, 49(1), 147-152. https://doi.org/10.5455/ajvs.221998.

Davis, A.N., Myers, W.A., Chang, C., Tate, B.N., Rico, J.E., Moniruzzaman, M., Haughey, N.J. and McFadden, J.W. 2021. Somatotropin increases plasma ceramide in relation to enhanced milk yield in cows. *Domestic Animal Endocrinology*, 74, 106480. https://doi.org/10.1016/j.domaniend.2020.

De Araujo Neto, F.R., Takada, L., Dos Santos, D.J.A., Aspilcueta-Borquis, R.R., Cardoso, D.F., do Nascimento, A.V., Leão, K.M., de Oliveira, H.N. and Tonhati, H. 2020. Identification of genomic regions related to age at first calving and first calving interval in water buffalo using single- step GBLUP. *Reproduction in Domestic Animals*, 55(11),1565-1572. https://doi.org/10.1111/rda.13811.

De Camargo, G.M.F., Aspilcueta-Borquis, R.R., Fortes, M.R.S., Porto-Neto, R., Cardoso, D.F., Santos, D.J.A., Lehnert, S.A., Reverter, A., Moore, S.S. and Tonhati, H. 2015. Prospecting major genes in dairy buffaloes. *Journal Biomedical Central Genomics*, 16, 1-14. https://doi.org/10.1186/s12864-015-1986-2.

Deng, T.X., Pang, C.Y., Liu, M.Q., Zhang, C. and Liang, X.W. 2016. Synonymous single nucleotide polymorphisms in the MC4R gene that are significantly associated with milk production traits in water buffaloes. *Genetics and Molecular Research*, 15, 1-8. https://doi.org/10.4238/gmr.15028153.

Deshmukh, B., Verma, A., Gupta, I.D., Kashyap, N. and Saikia, J. 2021. SNP identification in sperm associated antigen 11b gene and its association with sperm quality traits in Murrah bulls. *Indian Journal of Animal Research*, 55(9), 1005-1009. https://doi.org/10.18805/IJAR.B-4152.

Devkatte, S., Janmeda, M., Ramani, U., Patel, S., Dangar, N., Kharadi, V. and Pandya, G. 2021. Prolactin receptor gene exon 9 polymorphism in

Surti and Jaffarabadi buffaloes. *Indian Journal of Veterinary Sciences and Biotechnology*, 17(3), 36-40. https://doi.org/10.21887/ijvsbt.17.3.8.

Dhaware, S.A., Vaidya, M.S., Dongre, V.B. and Jadhav, P.V. 2024. Polymorphism of receptor genes of Follicle Stimulating Hormone (FSHR), Luteinizing Hormone (LHR) and Estrogen receptor alfa (era) in Marathwadi buffaloes. *Journal of Livestock Science*, 15(15), 307-313. https://doi.org/10.33259/JLivestSci.2024.307-313.

Dilbar, G.H., Qureshi, Z.I., Babar, M.E., Jamil, H. and Javed, M.T. 2019. R159Q Polymorphism in Leptin gene and its correlation with semen quality parameters in Nili-Ravi buffalo bulls. *Pakistan Veterinary Journal*, 39(1), 61-65. https://doi.org/10.29261/pakvetj/2018.122.

Druet, T., Fritz, S., Sellem, E., Basso, B., Gérard, O., Salas- Cortes, L., Humblot, P., Druart, X. and Eggen, A. 2009. Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. *Journal of Animal Breeding and Genetics,* 126(4), 269-277. https://doi.org/10.1111/j.1439-0388.2008.00788.x.

Easa, A.A., El-Aziz, A.H.A., Barbary, A.S.E., Kostomakhin, N.M., Nasr, M.A. and Imbabi, T.A. 2022. Genetic parameters of production and reproduction traits of Egyptian buffaloes under subtropical conditions. *Tropical Animal Health and Production*, 54(5), 270-275. https://doi.org/10.1007/s11250-022-03251-2.

EL-Awady, H., Shalaby, N.A. and Mourad, K.A. 2005. Variance componentsdue to direct and maternal effects and estimation of breeding values for some growth traits of Egyptian buffalo calves. *Journal of Animal and Poultry Production*, *Mansoura University Egypt*, 30(12), 7425-7436. https://doi.org/10.21608/jappmu.2005.238453.

EL-Awady, H.G., Salem, A.Y., Abdel-Glil, M.F., Zahed, S.M. and Abo El-Enin, A. 2017. Estimate of genetic and phenotypic trends for some productive and reproductive traits of Friesian cows in Egypt. *Journal of Animal and Poultry Production*, *Mansoura University Egypt*, 8(8), 329-334. https://doi.org/10.21608/jappmu.2017.45990.

El-Basuini, M.F. 2010. Genetic evaluation for semen traits on buffalo bulls. *M.Sc. Thesis, Faculty of Agriculture, Tanta University, Egypt*.

El Basuini, M.F., El Hais, A.A., Mourad, K.A.M., Awas, S.E.H.S. and Khattab, A.S. 2024. Genetic evaluation for milk and semen variables on Egyptian buffaloes. *Buffalo Bulletin*, 43(1), 45-50. https://doi.org/10.56825/bufbu.2024.431300.

El Faro, L. and Albuquerque, L.G.D. 2003. Estimation of genetic parameters for first lactation test-day records and total milk yield for Caracu cows. *Revista Brasileira de Zootecnia*, 32**,** 284-294. https://doi.org/10.1590/S1516-35982003000200006.

El Faro, L., Cardoso, V.L. and Albuquerque, L.G.D. 2008. Variance component estimates applying random regression models for test-day milk yield in Caracu heifers (Bos taurus Artiodactyla, Bovidae). *Genetics and Molecular Biology*, 31, 665-673. https://doi.org/10.1590/S1415-47572008000400011.

EL Nagar, A.G., Salem, M.M., Amin, A.M., Khalil, M.H., Ashour, A.F., Hegazy, M.M. and Abdel-Shafy, H. 2023. A single-step genome-wide association study for semen traits of Egyptian buffalo bulls. *Animals*, 13(24), 3758. https://doi.org/10.3390/ani1324375.

El-Arian, M.N., Shalaby, N.A., Khattab, A.S., Darwish, S.A. and Abou-Gamous, R.H. 2012. Phenotypic and genetic trends for some milk yield traits of Egyptian buffaloes. *Journal of Animal and Poultry Production, Mansoura University Egypt*, 3(7), 353-364. https://doi.org/10.21608/jappmu.2012.82938.

El-Bramony, M.M., 2011. Genetic and phenotypic parameters of milk yield and reproductive performance in the first three lactations of Egyptian buffalo. *Egyptian Journal of Animal Production*, 48(1), 1-10.

El-Bramony, M.M., 2014. Estimation of genetic and phenotypic parameters for milk yield, lactation length, calving interval and body weight in the first lactation of Egyptian buffalo. *Life Science Journal*, 11(12), 1012-1019.

El-Bramony, M.M., Nigm, A.A., Mourad, K.A., Ibrahim, M.A.M. and El-Saied, U.M. 2004. Estimation of genetic parameters for test day milk yield and somatic cell count in the first three lactations of Egyptian buffalo using random regression. In 12th Scientific Conference, Egyptian Society of Animal Production. *Mansoura University, Egypt*, 15-31.

El-Bramony, M.M., Ashmawy, A.A., Mourad, K.A. and El-Regalaty, H.A. 2017. Test-day milk, fat and protein yields as selection criteria in Egyptian buffalo. *Egyptian Journal of Animal Production*, 54(1), 1-10.

El-Debaky, H., El-Razik, K.A.A., Asa, S., Mahmoud, K.G.M., Kandie, M. and Ahmed, Y.F. 2020. Sequence based polymorphism of lactoferrin gene in relation to fertility in Egyptian buffaloes. *International Journal of Veterinary Science,* 9**,** 97-103.

El-Den, K., Mohammed, K. and Saudi, E. 2020. Estimation of genetic and non-genetic factors influencing growth traits performance of Egyptian buffalo. *Journal of Animal and Poultry Production, Mansoura University., Egypt*, 11(10), 383-388. https://doi.org/10.21608/jappmu.2020.123613.

El-Halawany, N., Shawky, A.E.M.A., M. Al-Tohamy, A.F., Hegazy, L., Abdel-Shafy, H., Abdel-Latif, M.A., Ghazi, Y.A., Neuhoff, C., Salilew-Wondim, D. and Schellander, K. 2017. Complement component 3: characterization and association with mastitis resistance in Egyptian water buffalo and cattle. *Journal of Genetics*, 96, 65-73. https://doi.org/10.1007/s12041-017-0740-8.

EL-Hedainy, D.K.E.H., Elbanhawy, K.E.A., Amin, A., Salem, M.M., Hammoud, M.H. and El-Barbary, A.S. 2020. Genetic trend for milk production and longevity traits of Egyptian buffalo. *Egyptian Journal of Animal Production,* 57(3), 95-100.

El-Komey, S.M., Hussein, K., Badr, A.A. and Khattab, A.S. 2016. Single and Multi Traits Animal Models for some Semen Traits on Friesian

Bulls. *Journal of Animal and Poultry Production*, 7(7), 265-268. https://doi.org/10.21608/jappmu.2016.48711.

El-Komy, S.M., Saleh, A.A., Abdel-Hamid, T.M. and El-Magd, M.A. 2020. Association of GHR polymorphisms with milk production in buffaloes. *Animals*, 10(7), 1203. https://doi.org/10.3390/ani10071203.

El-Magd, M.A., Abo-Al-Ela, H.G., El-Nahas, A., Saleh, A.A. and Mansour, A.A., 2014. Effects of a novel SNP of IGF2R gene on growth traits and expression rate of IGF2R and IGF2 genes in gluteus medius muscle of Egyptian buffalo. *Gene*, 540(2), 133-139. https://doi.org/10.1016/j.gene.2014.02.059.

El-Magd, M.A.E., Zaki, A.G., Kahilo, K.A., Barakat, M.E.S. and Hassan, I.F. 2015. Effect of SNPs in prolactin promoter on milk traits in Egyptian buffalo. *Journal Advances in Dairy Research*, 3(128), 2. https://doi.org/10.4172/2329-888X.1000128.

El-Magd, M.A., Saleh, A.A., Nafeaa, A.A., El-Komy, S.M. and Afifi, M.A. 2017. Polymorphisms of the IGF1 gene and their association with growth traits, serum concentration and expression rate of IGF1 and IGF1R in buffalo. *Journal of Zhejiang University. Science*. B 18(12), 1064-1074. https://doi.org/10.1631/jzus.B1600573.

El-Magd, M.A., Fathy, A., Kahilo, K.A., Saleh, A.A., El Sheikh, A.I., Al-Shami, S. and El-Komy, S.M. 2021. Polymorphisms of the PRLR gene and their association with milk production traits in Egyptian buffaloes. *Animals*, 11(5), 1237. https://doi.org/10.3390/ani11051237.

Elsayed, M., Al-Momani, A.Q., Amin, M.J. and Al-Najjar, K.A. 2021. Genetic, phenotypic, and environmental trends towards improving birth and weaning weights of Syrian buffalo calves. *Journal of Livestock Science*, (12), 76-84. https://doi.org/10.33259/JLivestSci.2021.76-84.

Erdoğan, M., Tekerli, M., Çelikeloğlu, K., Hacan, Ö., Kocak, S., Bozkurt, Z., Cinkaya, S. and Demirtaş, M. 2021. Associations of SNPs in GHR gene with growth and milk yield of Anatolian buffaloes. *Turkish Journal of Veterinary & Animal Sciences*, 45(6), 1080-1086. https://doi.org/10.3906/vet-2103-40.

Erhardt, G. and Weimann, C. 2007. Use of molecular markers for evaluation of genetic diversity and in animal production. *Archivos Latinoamericanos de Producción Animal*, 15(5), 63-66.

Eriani, K., Rahmi, R., Jamil, I., Rosnizar, R. and Azhar, A.L. 2019. Body size characteristics and polymorphism in *GH* and GHRH genes of Simeulue buffalo of Aceh, Indonesia. *Biodiversitas Journal of Biological Diversity*, 20(1), 236-242. https://doi.org/10.13057/biodiv/d200127.

Falleiro, V., Silveira, E.S., Ramos, A.A., Carneiro, P.L.S., Carrillo, J.A. and Malhado, C.H.M. 2013. Genetic parameters for growth traits of Mediterranean buffaloes from Brazil, estimated by Bayesian inference. *Editorial Board*, 32, 627-931.

Fooda, T.A., Mourad, K.A. and Gebreel, I.A. 2010. Phenotypic and genetic trends for milk production in Egyptian buffaloes. *Journal of American Science*, 6(11), 143-147.

Fouda, M., Hemeda, S., El-Bayomi, K., El-Araby, I., Hendam, B. and Ateya, A. 2021. Genetic polymorphisms in FSHR/ALUI and ESRα/BG1I loci and their association with repeat breeder incidence in buffalo. *Journal of the Hellenic Veterinary Medical Society*, 72(2), 2869-2878. https://doi.org/10.12681/jhvms.

Freeman, M.E., Kanyicska, B., Lerant, A. and Nagy, G. 2000. Prolactin: structure, function, and regulation of secretion. *Physiological Reviews***.** 80, 1523. https://doi.org/10.1152/physrev.2000.80.4.

Freitas, A.C., De Camargo, G.M.F., Aspilcueta-Borquis, R.R., Stafuzza, N.B., Venturini, G.C., Tanamati, F., Hurtado-Lugo, N.A., Barros, C.C. and Tonhati, H. 2016. Polymorphism in the A2M gene associated with high-quality milk in Murrah buffaloes (Bubalus bubalis). *Genetics and Molecular Research*, 15(10). https://doi.org/10.4238/gmr.15027637.

Gafer, J.A., El-Rahman, G.H.A. and Rawash, Z.M. 2015. Association of HSP70 gene polymorphism and bull semen quality in winter and

summer seasons. *Alexandria Journal of Veterinary Sciences*, 46(1), 1-14. https://doi.org/10.5455/ajvs.186038.

Geetha, E., Chakravarty, A.K. and Kumar, K.V. 2006. Genetic persistency of first lactation milk yield estimated using random regression model for Indian Murrah buffaloes. *Asian-australasian Journal of Animal Sciences*, 19(12), 1696-1701. https://doi.org/10.5713/ajas.2006.1696.

Ghiasi, H. and Honarvar, M. 2016. Genetic and phenotypic trends of fertility traits in Iranian Holstein cows. *Iranian Journal of Applied Animal Science*, 6(1), 53-58.

Gil, F.M.M., De Camargo, G.M.F., de Souza, F.P., Cardoso, D.F., Fonseca, P.D.S., Zetouni, L., Braz, C.U., Aspilcueta-Borquis, R.R. and Tonhati, H. 2013. Polymorphisms in the GHRELIN gene and their associations with milk yield and quality in water buffaloes. *Journal of Dairy Science*, 96(5), 3326-3331. https://doi.org/10.3168/jds.2012-6362.

Gowane, G.R. and Vohra, V. 2022. Advances in Buffalo Breeding: A Journey from Classical Breeding to Genomic Selection. *In Biotechnological Applications in Buffalo Research* (57-77). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-16-7531-7.

Gregerson, K. 2006. Prolactin: Structure, Function, and Regulation of Secretion. *Knobil Neill's Physiol. Reprod*. 1703–1726. https://doi.org/10.1152/physrev.2000.80.4.1523.

Groeneveld, E. 2006. PEST User's Manual. Farm Animal Genetics, Neustadt, Germany.

Groeneveld, E. 2010. VCE user's guide and reference manual version 6.0. *ftp://ftp. tzv. fal. de/pub/vce6/doc/vce6-manual-3.1-A4. pdf.*

Gupta, J.P., Sachdeva, G.K., Gandhi, R.S. and Chakaravarty, A.K. 2015. Developing multiple-trait prediction models using growth and production traits in Murrah buffalo.. *Buffalo Bulletin*, 34(3), 347-355.

Guzman, J.L.G., Lázaro, S.F., do Nascimento, A.V., de Abreu Santos, D.J., Cardoso, D.F., Scalez, D.C.B., de Albuquerque, L.G., Lugo, N.A.H. and Tonhati, H. 2020. Genome-wide association study applied

to type traits related to milk yield in water buffaloes (Bubalus bubalis). *Journal of Dairy Science*, 103(2), 1642-1650. https://doi.org/10.3168/jds.2019-16499.

Hasanain, M.H., Mahmoud, K.G.M., El-Menoufy, A., Sakr, A., Ahmed, Y. and Othman, O. 2016. Semen characteristics and genotyping of pituitary-specific transcription factor gene in buffalo using PCR-RFLP. *Egyptian Journal of Veterinary Sciences*, 47(1),13-26. https://doi.org/10.21608/ejvs.2016.1079.

Hasanain, M.H., Mahmoud, K.Gh.M., Ahmed, Y.F., EL-Menoufy, A.A., Sakr, A.M., and Othman. E.O. 2017. Effect of body condition score and PCR-RFLP polymorphism of prolactin gene on semen characteristics of buffalo bulls (BubalusBubalis). *Egyptian Journal of Veterinary Sciences*, 48(1),1-9. https://doi.org/10.21608/ejvs.2017.868.1011.

Helmy, A.A.H. and Somida, R.A. 2021. Genetic parameters for some reproductive traits in Egyptian buffaloes. *Egyptian Journal of Animal Production*, 58(2), 57-61. https://doi.org/10.21608/ejap.2021.67104.1011.

Herrera, A.C., Múnera, O.D. and Cerón-Muñoz, M.F. 2013. Variance components and genetic parameters for milk production of Holstein cattle in Antioquia (Colombia) using random regression models. *Revista Colombiana de Ciencias Pecuarias*, 26(2), 90-97.

Heydarian, D., Miraei-Ashtiani, S.R. and Sadeghi, M. 2014. Study on DGAT1-exon8 polymorphism in Iranian buffalo. *International Journal of Advanced Biological and Biomedical Research*, 2(7), 2276-2282.

Hosseini, S.M., Tingzhu, Y., Pasandideh, M., Liang, A., Hua, G., Farmanullah, Schreurs, N.M., Raza, S.H.A., Salzano, A., Campanile, G. and Gasparrini, B. 2021. Genetic association of PPARGC1A gene single nucleotide polymorphism with milk production traits in Italian Mediterranean buffalo. *Biomed Research International*, (1), 3653157. https://doi.org/10.1155/2021/3653157.

Hu, X., Lü, A., Chen, H., Gao, X., Xu, H., Zhang, C., Fang, X. and Lei, C. 2009. Preliminary evidence for association of prolactin and prolactin receptor genes with milk production traits in Chinese Holsteins. *Journal of Applied Animal Research*, 36(2), 213-217. https://doi.org/10.1080/09712119.2009.9707062.

Hurtado-Lugo, N., Cerón-Muñoz, M. and Gutiérrez-Valencia, A. 2006. Estimates of genetic parameters of milk yield in test day analysis in buffaloes of the Colombian Atlantic Coast. *Livestock Research for Rural Development*, 18(3), 39.

Hurtado-Lugo, N., Cerón-Muñoz, M., Tonhati, H., Bignardi, A., Restrepo, L. and Aspilcuelta, R. 2009. Estimates of genetic parameters for Test-day using a random regression model for first lactations of buffalo in Colombia North Coast. *Livestock Research for Rural Development*, 21(6), 89.

Iamartino, D., Williams, J.L., Sonstegard, T., Reecy, J., Tassell, C.V., Nicolazzi, E.L., Biffani, S., Biscarini, F., Schroeder, S., Oliveira, D.D. and Coletta, A. 2013. The buffalo genome and the application of genomics in animal management and improvement.*Buffalo Bulletin*, 32, 151-158.

Ibrahim, M.A.M., Rushdi, H.E., Abdel-Salam, S.A.M. and Abou-Bakr, S. 2009. Genetic and phenotypic trends of calving interval and age at first calving in a commercial Holstein herd. *Egyptian Journal of Animal Production*, 46(2), 103-112.

Ibrahim, M.A., Khattab, A.S., El-Habaeib, S., Awad, S. and Tőzsér, J. 2012. Genetic parameters for buffalo milk yield and milk quality traits using animal model. *Animal Welfare, Ethology and Housing Systems*, 8 (2), 175-182. https://doi.org/10.3168/jds.2009-2621.

Ilatsia, E.D., Muasya, T.K., Muhuyi, W.B. and Kahi, A.K. 2007. Genetic and phenotypic parameters and annual trends for milk production and fertility traits of the Sahiwal cattle in semi arid Kenya. *Tropical Animal*

Health and Production, 39, 37-48. https://doi.org/10.1007/s11250-006-4408-2.

Intaratham, W., Koonawootrittriron, S., Sopannarath, P., Graser, H.U. and Tumwasorn, S. 2008. Genetic parameters and annual trends for birth and weaning weights of a Northeastern Thai indigenous cattle line. *Asian-Australasian Journal of Animal Sciences*, 21(4), 478-483. https://doi.org/10.5713/ajas.2008.70149.

Ishaq, R., Suleman, M., Riaz, M.N., Yousaf, M., Shah, A. and Ghafoor, A. 2013. Prolactin gene polymorphism in Nili- Ravi buffaloes in relation to Sahiwal and Achai Cattle. *International Journal of Dairy Technology*, 66(1), 20-24. https://doi.org/ 10.1111/j.1471-0307.2012.00875.

Jamrozik, J. and Schaeffer, L.R. 1997. Estimates of genetic parameters for a test day model with random regressions for yield traits of first lactation *Holsteins. Journal of Dairy Science*, 80(4), 762-770. https://doi.org/10.3168/jds.S0022-0302(97)75996-4.

Jamrozik, J., Schaeffer, L.R. and Jansen, G.B. 2000. Approximate accuracies of prediction from random regression models. *Livestock Production Sciences*, 66, 85-92.

Jamuna, V., Gupta, A.K., Chakravarty, A.K., Singh, A., Patil, C.S., Kumar, M. and Vohra, V. 2016. Leptin gene polymorphism in association with lactation milk yield in Murrah buffaloes. *The Indian Journal of Animal Sciences*, 86(1), 95-97. https://doi.org/10.56093/ijans.v86i1.55039.

Kadhim, A.F. and Ibrahim, W.I. 2019. Polymorphic genotypes for fragment (Intron1-661 bp) from *DGAT1* gene and their relationship to performance in Iraqi buffalo using RELP technology. *Journal Plant Archives*, 19, 4032-4036.

Kadoom, A.K., El-Keraby, F.E., Ashour, A.F. and El-Siefy, E.M. 2016. Seminal plasma proteins as potential markers of semen characteristics in buffalo and bovine bulls. *Journal of Animal and Poultry Production*, 7(8), 287-291. https://doi.org/10.21608/jappmu.2016.48715.

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

Kaplan, S. 2018. Nucleotide polymorphism of leptin gene in Anatolian Water buffaloes. *Pakistan Journal of Zoology*, 50(5), 1841-1846. https://doi.org/10.17582/journal.pjz/2018.50.5.1841.1846.

Kaplan, Y. and Tekerli, M. 2023. Genetic parameters of growth, reproductive, and productive characteristics in Anatolian buffaloes. *Turkish Journal of Veterinary & Animal Sciences*, 47(3), 222-228. https://doi.org/10.55730/1300-0128.4289.

Kapš, M., Posavi, M., Stipić, N. and Mikulić, B. 2000. Genetic evaluation of semen and growth traits of young Simmental bulls in performance test. *Agriculturae Conspectus Scientificus*, 65(1), 15-20. https://doi.org/hrcak.srce.hr/12507.

Kathiravan, R., Chitra, R., Murali, N. and Arthanarieswaran, M., 2019. Detection of follicle stimulating hormone receptor gene polymorphism in Murrah and graded Murrah buffaloes. *International Journal of Livestock Research*, 9(7), 143-147. https://doi.org/10.5455/ijlr.201811130600.

Kgari, R.D., Muller, C.J.C., Dzama, K. and Makgahlela, M.L., 2023. Genetic and phenotypic trends for female fertility traits derived from service records of South African Holstein cattle. *South African Journal of Animal Science*, 53(4), 598-606. https://doi.org/10.4314/sajas.v53i4.14.

Khan, M.S. 1998. Animal model evaluation of Nili-Ravi buffaloes. *In Proc. 6th World Genetics Applied to Livestock Production., Armidale, NSW, Australia*, 24, 467-470.

Khan, M.Z., Ma, Y., Ma, J., Xiao, J., Liu, Y., Liu, S., Khan, A., Khan, I.M. and Cao, Z. 2021. Association of *DGAT1* with cattle, buffalo, goat, and sheep milk and meat production traits. *Frontiers in Veterinary Science*, 8, 712470. https://doi.org/10.3389/fvets.2021.712470.

Khan, A., Singh, K., Jaiswal, S., Raza, M., Jasrotia, R.S., Kumar, A., Gurjar, A.K.S., Kumari, J., Nayan, V., Iquebal, M.A. and Angadi, U.B. 2022. Whole-genome-based web genomic resource for water buffalo (Bubalus bubalis). *Frontiers in Genetics*, 13, 809741. https://doi.org/10.3389/fgene.2022.809741.

Khan, M.Z., Chen, W., Naz, S., Liu, X., Liang, H., Chen, Y., Kou, X., Liu, Y., Ashraf, I., Han, Y. and Peng, Y. 2024. Determinant genetic markers of semen quality in livestock. *Frontiers in Endocrinology*, 15, 1456305. https://doi.org/10.3389/fendo.2024.1456305.

Khattab, A.S. and Mourad, K.A. 1992. Estimation of genetic parameters and genetic trends for some milk traits in a herd of Egyptian buffaloes. *Egyption Journal Animal Prodation*, 29, 33-44.

Khattab, A.S., El-Awady, H.G., El-Arian, M.N. and Mourad, K.A. 2003. Genetic analysis of some performance traits using an animal model in a herd of Egyptian buffaloes. *Egyptian Journal of Animal Production*, 40(1), 15-26.

Khattab, A.S., Mourad, A. and Awad, S., 2010. Estimation of genetic parameters and breeding values for some productive traits on Egyptian buffaloes. Revista Veterinaria, 21(1).

Khattab, A.S., Zeweil, H.S., Ahmed, M.H., Asmaa, Z.M. and Mourd, K.A. 2015. A comparison of different selection indexes for some semen traits on Egyptian buffalo bulls. *Middle East Journal of Applied Sciences*, 5(02), 368-373.

Khattab, A.S., Adenaike, A.S., Shymma, M.E.K., El-Gendy, M.M., Peters, S.O. and Hussein, A.M. 2022. Genetic evaluation of semen traits in Friesian bulls raised in Egypt. *Theriogenology*, 179, 39-44. https://doi.org/10.1016/j.theriogenology.2021.11.011.

Koetz Júnior, C., Roso, V.M., Claus, L.A.M., Oliveira, R.P.L.D., Fávaro, P.D.C., Borges, M.H.F., Barcellos, J.O.J. and Ribeiro, E.L.D.A. 2017. Genetic trends for mature body weight, visual scores, and growth traits in Nellore cattle. *Ciência Rural*, 47, e20151506. https://doi.org/10.1590/0103-8478cr20151506.

Konca, M.A. and Akyüz, B. 2017. Investigation of growth hormone releasing hormone, growth hormone and prolactin hormone gene polymorphism in Anatolian water buffalo. *Annals of Animal Science*, 17, 1053-1062. https://doi.org/10.1515/aoas-2016-0100.

Korhonen, T. 1996. "The dairy cattle evaluation of 1996". *http://www.mloy.fi/faba/blup/blup1.html,23.01.1977 (h:14:13) (Article)*.

Kour, G. and Narang, R. 2021. Estimates of genetic parameters of economic traits in Murrah buffaloes: Genetic parameters of economic traits in Murrah buffaloes. *Journal of Agriculture Search*, 8(2), 173-176. https://doi.org/10.21921/jas.v8i2.7304.

Kour, G., Kour, H., Singh, B., Singh, P.K. and Neeraj, A. 2021. Influence of non-genetic factors on performance traits in Murrah buffaloes: A review. The Pharma Innovation Journal,10(7): 265-269.

Krovvidi, S., Aranganoor, T.K., Ramasamy, S., Regula, V., Metta, M. and Mani, J. 2021. Evaluation of CYP19 as a candidate gene for milk production traits in native cattle and buffalo populations of Southern India. *Turkish Journal of Veterinary & Animal Sciences*, 45(5), 863-872. https://doi.org/10.3906/vet-2101-4.

Kumar, V. and Chakravarty, A.K. 2016. Test-day genetic analysis of Murrah buffalo sires for milk production. *Buffalo Bulletin*, 35(1), 59-64.

Kumar, V. and Chakravarty, A.K., 2016. Evaluation of breeding values Murrah buffalo bulls under organized farms. *Buffalo Bulletin*, 35(3), 371-377.

Kumar, V., Chakravarty, A.K., Patil, C.S., Jamuna, V., Mahajan, A., Shivahre, P.R. and Magotra, A. 2014. Application of monthly test-day milk yield in Murrah buffalo. *Indian Journal of Animal Sciences*, 84(11), 1202-1205. https://doi.org/10.56093/ijans.v84i11.44779.

Kumar, V., Chakravarty, A.K., Patil, C.S., Valsalan, J. and Mahajan, A., 2015. Estimate of genetic and non-genetic parameters for age at first calving in Murrah buffalo. The Indian Journal of Animal Sciences, 85(1), 84-85. https://doi.org/10.56093/ijans.v85i1.46193.

Kumar, M., Vohra, V., Ratwan, P., Valsalan, J., Patil, C.S. and Chakravarty, A.K. 2016. Estimates of genetic parameters for fat yield in Murrah buffaloes. *Veterinary World*, 9(3), 295-298. https://doi.org/10.14202/vetworld.2016.295-298.

Kumar, M., Vohra, V., Ratwan, P., Chopra, A. and Chakaravarty, A.K. 2017. Influence of FASN gene polymorphism on milk production and its composition traits in Murrah buffaloes. *Indian Journal of Animal Research*, 51(4), 640-643. https://doi.org/10.18805/ijar.7077.

Kumar, D., Mehta, J.S., Jerome, A., Kumar, P., Kumar, D., Shivani, B., Patil, C.S., Bala, R., Verma, N., Nain, S. and Sharma, R.K. 2023. Genetic analysis for semen quality traits in buffalo bulls. *Tropical Animal Health and Production*, 55(5), 313. https://doi.org/10.1007/s11250-023-03756.

Kumari, R., Ramesha, K.P., Kumar, R., Divya, P., Kumari, A., Sinha, B. and Gonge, D.S. 2018. Sequence characterization and polymorphism detection in AQP7 gene of Murrah buffalo. *Indian Journal of Animal Research*, 52(3), 378-382. https://doi.org/10.18805/ijar.v0iOF.4549.

Kunaka, K. and Makuza, S. M. 2005. Genetic and environmental trends for milk traits in the Zimbabwean Holstein-Friesian Population. Pakistan *Journal Biological Sciences*, 8(7), 1011-1015.

Kuralkar, S.V. and Raheja, K.L. 1997. Estimation of genetic and phenotypic trends for first lactation traits in Murrah buffaloes. *The Indian Journal of Animal Sciences*, 67(8), 695-697.

Kuralkar, S.V. and Raheja, K.L. 2001. Estimation of genetic and phenotypic trends for first dry period and milk efficiency traits in Murrah buffaloes. *Indian Journal of Veterinary Research*, 10 (1), 7-13.

Ladani, D.D., Pipalia, D.L., Brahmkshtri, B.P., Rank, D.N., Joshi, C.G., Vataliya, P.H. and Solanki, J.V. 2003. Prolactin genotyping of Indian buffalo breeds using PCR-RFLP. *Buffalo Journal*, 19(2), 203-208.

Lázaro, S.F., Tonhati, H., Oliveira, H.R., Silva, A.A., Scalez, D.C., Nascimento, A.V., Santos, D.J., Stefani, G., Carvalho, I.S.,

Sandoval, A.F. and Brito, L.F. 2024. Genetic parameters and genome-wide association studies for mozzarella and milk production traits, lactation length, and lactation persistency in Murrah buffaloes. *Journal of Dairy Science*, 107(2), 992-1021. https://doi.org/10.3168/jds.2023-23284.

Lazebnaya, I.V., Lazebny, O.E., Khatami, S.R. and Sulimova, G.E. 2013. Use of the bovine prolactin gene (bprl) for estimating genetic variation and milk production in Aboriginal russian breeds of Bos taurus l. in prolactin. IntechOpen. 35–52. https://doi.org/10.5772/54756.

Le Provost, F., Leroux, C., Martin, P., Gaye, P. and Djiane, J. 1994. Prolactin gene expression in ovine and caprine mammary gland. *Neuroendocrinology*, 60(3), 305-313. https://doi.org/10.1159/000126763.

Lechniak, D., Machnik, G., Szydlowski, M. and Switonski, M. 1999. Growth hormone gene polymorphism and reproductive performance of AI bulls. *Theriogenology*, 52(7), 1145-1152. https://doi.org/10.1016/S0093-691X(99)00206.

Legarra, A., L. Varona, and E. de Maturana. 2008. TM Threshold model. Retrieved on 26, 2008.

Li, J., Liang, A., Li, Z., Du, C., Hua, G., Salzano, A., Campanile, G., Gasparrini, B. and Yang, L. 2017. An association analysis between *PRL* genotype and milk production traits in Italian Mediterranean river buffalo. *Journal of Dairy Research*, 84(4), 430-433. https://doi.org/10.1017/S0022029917000693.

Li, J., Liu, J., Campanile, G., Plastow, G., Zhang, C., Wang, Z., Cassandro, M., Gasparrini, B., Salzano, A., Hua, G. and Liang, A. 2018a. Novel insights into the genetic basis of buffalo reproductive performance. *BMC genomics*, 19, 1-11. https://doi.org/10.1186/s12864-018-5208-6.

Li, J., Liu, J., Liu, S., Plastow, G., Zhang, C., Wang, Z., Campanile, G., Salzano, A., Gasparrini, B., Hua, G. and Liang, A. 2018b.

Integrating RNA-seq and GWAS reveals novel genetic mutations for buffalo reproductive traits. *Animal Reproduction Science*, 197, 290-295. https://doi.org/10.1016/j.anireprosci.2018.08.041.

Li, J., Liu, S., Li, Z., Zhang, S., Hua, G., Salzano, A., Campanile, G., Gasparrini, B., Liang, A. and Yang, L., 2018c. *DGAT1* polymorphism in Riverine buffalo, Swamp buffalo and crossbred buffalo. *Journal of Dairy Research*, 85(4), 412-415. https://doi.org/10.1017/S0022029918000468.

Liu, Y.X., Zhang, J., Schaeffer, L.R., Yang, R.Q. and Zhang, W.L. 2006. Optimal random regression models for milk production in dairy cattle. *Journal of Dairy Science*, 89(6), 2233-2235. https://doi.org/10.3168/jds.S0022-0302(06)72294-9.

Liu, J., Wang, Z., Li, J., Li, H. and Yang, L. 2020. Genome-wide identification of Diacylglycerol Acyltransferases (DGAT) family genes influencing milk production in buffalo. *BMC Genetics*, 21, 1-14. https://doi.org/10.1186/s12863-020-0832.

Lü, A., Hu, X., Chen, H., Dong, Y., Zhang, Y. and Wang, X. 2011. Novel SNPs of the bovine PRLR gene associated with milk production traits. *Biochemical Genetics*, 49, 177-189. https://doi.org/10.1007/s10528-010-9397-1.

Lu, G., He, C., Yang, X., Cheng, Y., Yang, R., Song, J., Wang, S., Wang, C., Ma, Z., Li, J. and Feng, T. 2022. Association between single-nucleotide polymorphisms in ARID4A gene and sperm quality of Chinese water buffalo. Veterinarski Arhiv, 92(5), 577-590. https://doi.org/10.24099/vet.arhiv.1736.

Ma, X., Liang, S., Liang, A., Rushdi, H.E. and Deng, T., 2021. Evolutionary analysis of OAT gene family in river and swamp buffalo: potential role of SLCO3A1 gene in milk performance. *Genes*, 12(9), 1394. https://doi.org/10.3390/genes12091394.

Machado, E., Yoshimura, E.H., Santos, N.W., Agustinho, B.C., Pereira, L.D.M., Samensari, R.B., de Aguiar, S.C. and Zeoula, L.M. 2016a. Nitrogen metabolism, digestive parameters, and protein requirements

for the maintenance of buffalo growth. *Tropical Animal Health and Production*, 48, 361-366. https://doi.org/10.1007/s11250-015-0959-4.

Machado, E.B., Souza, B.B., Guimarães, R.C., Azevedo, J.S.N., Gonçalves, E.C., Ribeiro, H.F.L., Filho, S.R. and Filho, E.S. 2016b. Polymorphism in the melatonin receptor gene in buffalo populations of the Brazilian Amazon. *Journal of Genetics and Molecular Research*, 15, 1-6. https://doi.org/10.4238/gmr.15027960.

Madad, M., Ghavi Hossein-Zadeh, N., Shadparvar, A.A. and Kianzad, D. 2013. Random regression models to estimate genetic parameters for test-day milk yield and composition in Iranian buffaloes. *Archives Animal Breeding*, 56(1), 276-284. https://doi.org/10.7482/0003-9438-56-027.

Madnalwar, V.S., Sawane, M.P., Pawar, V.D., Patil, P.A., Fernandis, A.P. and Bannalikar, A.S., 2010. Genotyping the prolactin gene in Pandharpuri buffaloes by PCR-RFLP. Buffalo Bulletin, 29(2), 88-91.

Mahdy, A.E., El-Shafie, O.M. and Ayyat, M.S. 1999. Genetic study and sire values for some economic traits in Egyptian buffaloes. *Alexandria Journal of Agricultural Research*, 44(2), 15-35.

Mahmoud, K.G., Sakr, A.M., Ibrahim, S.R., Sosa, A.S., Hasanain, M.H. and Nawito, M.F. 2021. GnRHR gene polymorphism and its correlation with semen quality in Buffalo bulls (Bubalus bubalis). *Iraqi Journal of Veterinary Sciences*, 35, 381-386.

Mahrous, K.F., Aboelenin, M.M., Rashed, M.A., Sallam, M.A. and Rushdi, H.E. 2020. Detection of polymorphism within leptin gene in Egyptian river buffalo and predict its effects on different molecular levels. *Journal of Genetic Engineering and Biotechnology*, 18(1), 6. https://doi.org/10.1186/s43141-020-0020-5.

Malhado, M., Ramos, A., Carneiro, S., De Souza, J.C. and Lamberson, W.R. 2007. Genetic and phenotypic trends for growth traits of buffaloes in Brazil. *Italian Journal of Animal Science*, 6(sup2), 325-327. https://doi.org/10.4081/ijas.2007.s2.325.

Malhado, M.C.H., Mendes Malhado, A.C., Amorim Ramos, A., Souza Carneiro, P.L., Siewerdt, F. and Pala, A. 2012. Genetic parameters by Bayesian inference for dual purpose Jaffarabadi buffaloes. *Archives Animal Breeding*, 55(6), 567-576. https://doi.org/10.5194/aab-55-567-2012.

Marques, J.R.F., Júnior, J.B.R., Carvalho, L.D.M., Nascimento, C.N.B., Costa, N.D., Barbosa, C., Batista, H.A.M., Ribeiro, M.J.P.S., Ramos, A.A. and Pimentel, E.S. 1991. Genetic parameters of productive traits in dairy buffaloes (Bubalus bubalis L.). *Bolteim de Pesquisa - Centro de Pesquisa Agropecuaria do Tropico Umido*, 40, 123-126. https://doi.org/10.1016/S2352-4642(21)00286-8.

Marson, E.P., Ferraz, J.B.S., Meirelles, F.V., Balieiro, J.C.D.C. and Eler, J.P. 2008. Effects of polymorphisms of LHR and *FSHR* genes on sexual precocity in a Bos taurus x Bos indicus beef composite population. *Genetics and Molecular Research*, 7(1), 243-251. https://doi.org/10.4238/vol7-1gmr418.

Mathevon, M., Buhr, M.M. and Dekkers, J.C.M. 1998. Environmental, management, and genetic factors affecting semen production in Holstein bulls. *Journal of dairy science*, 81(12), 3321-3330. https://doi.org/10.3168/jds.S0022-0302(98)75898-9.

Maudet, C., Luikart, G. and Taberlet, P. 2002. Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis. *Journal of Animal Science*, 80(4), 942-950. https://doi.org/10.2527/2002.804942x.

Mavi, G.K., Dubey, P.P., Kumar, B.S., Kaur, S., Malhotra, P. and Narang, R. 2017. Allele fixation in prolactin and Pit-1 genes associated with highest fat content than milk yield of Murrah buffalo population. *Journal of Animal Research*, 7(6), 993-1000. https://doi.org/10.5958/2277-940X.2017.00150.

Meyer, K. 1998. Modeling 'repeated'records: covariance functions and random regression models to analyse animal breeding data. *In*

Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, (25), 517-520.

Meyer, K. 2004. Scope for a random regression model in genetic evaluation of beef cattle for growth. *Livestock Production Science*, 86(1-3), 69-83. https://doi.org/10.1016/S0301-6226(03)00142-8.

Meyer, K. and Hill, W.G. 1997. Estimation of genetic and phenotypic covariance functions for longitudinal or 'repeated'records by restricted maximum likelihood. *Livestock Production Science*, 47(3), 185-200. https://doi.org/10.1016/S0301-6226(96)01414-5.

Meyer, L., Powell, J., Kutz, B., Looper, M., Brown Jr, A.H. and Rosenkrans Jr, C. 2017. Associations of single nucleotide polymorphisms in the bovine prolactin gene with phenotypic traits in beef cattle. *Agriculturei Genetic*, 5, 7-11. https://doi.org/10.1016/j.aggene.2017.06.002.

Minervino, A.H.H., Zava, M., Vecchio, D. and Borghese, A. 2020. Bubalus bubalis: A short story. Frontiers in veterinary science, 7, 570413.

Minj, A., Mondal, S., Tiwari, A.K., Sharma, B. and Varshney, V.P. 2008. Molecular characterization of follicle stimulating hormone receptor (FSHR) gene in the Indian river buffalo (Bubalus bubalis). General and Comparative Endocrinology, 158(2), 147-153. https://doi.org/10.1016/j.ygcen.2008.07.003.

Mishra, B.P., Dubey, P.K., Prakash, B., Kathiravan, P., Goyal, S., Sadana, D.K., Das, G.C., Goswami, R.N., Bhasin, V., Joshi, B.K. and Kataria, R.S. 2015. Genetic analysis of river, swamp and hybrid buffaloes of north- east India throw new light on phylogeography of water buffalo (Bubalus bubalis). *Journal of Animal Breeding and Genetics*, 132(6), 454-466. https://doi.org/10.1111/jbg.12141.

Misra, S.S., Arjava Sharma, A.S., Bhattacharya, T.K., Kumar, P. and Roy, S.S. 2008. Association of breed and polymorphism of αS1-and αS2-casein genes with milk quality and daily milk and constituent yield traits of buffaloes (Bubalus bubalis). *Buffalo Bulletin*, 27**,** 294-301.

Misztal, I., Tsuruta, S., Lourenco, D.A.L., Masuda, Y., Aguilar, I., Legarra, A. and Vitezica, Z. 2018. Manual for BLUPF90 family programs. University of Georgia. *Athens, USA*, 125.

Mohamed, S.A.M., Mostafa, H.A., Abdel-Aziz, M.M., Abdel-Salam, S.A.M., Ibrahim, M.A.M. and Sadek, R.R. 2010. Estimation of genetic and phenotypic trend of total milk yield of Egyptian buffalo raised at experimental farm in Egypt. *Journal of Animal and Poultry Production*, Mansoura University, 1(7), 275-281. https://doi.org/10.21608/jappmu.2010.86228.

Morammazi, S., Torshizi, R., Rouzbehan, Y. and Sayyadnejad, M.B. 2007. Estimates of genetic parameters for production and reproduction traits in Khuzestan buffalos of Iran. *Italian Journal of Animal Science*, 6(Sup2), 421-424. https://doi.org/10.4081/ijas.2007.s2.421.

Mostafa, M.A., Darwish, S.A. and El-Fatah, A. 2017. Estimation of genetic parameters for some production and reproduction traits using different animal models in Egyptian buffaloes. *Journal of Animal and Poultry Production*, Mansoura University, 8(2), 21-25. https://doi.org/10.21608/jappmu.2017.45751.

Mourad, K.A.M. and Khattab, A.S. 2009. A comparison between different selection indices for some productive traits on Egyptian buffaloes. *Archives Animal Breeding*, 52(5), 476-484. https://doi.org/10.5194/aab-52-476-2009.

Nadeem, A. and Maryam, J. 2016. Genetic and genomic dissection of prolactin revealed potential association with milk production traits in Riverine buffalo. *Tropical Animal Health and Production*, 48, 1261-1268. https://doi.org/10.1007/s11250-016-1086-6.

Nafiu, L.O., Muzuni, M. A., Pagala, W. and Kurniawan dan S. Rahadi. 2020. Identification of growth genes diversity of swamp buffalo using RFLP in Kabaena Island, Bombana District, Southeast Sulawesi, Indonesia. *Biodiversitas Journal of Biological Diversity*, 21(5), 1901–1907. https://doi.org/10.13057/biodiv/d210516.

Nazari, M.A., Ghavi Hossein-Zadeh, N., Shadparvar, A.A. and Kianzad, D. 2021. Genetic analysis of persistency for Milk fat yield in Iranian buffaloes (Bubalus bubalis). *Frontiers in Genetics*, 12, 633017. https://doi.org/10.3389/fgene.2021.633017.

Nikitkina, E., Krutikova, A., Musidray, A. and Plemyashov, K. 2021. Search for associations of FSHR, INHA, INHAB, PRL, TNP2 and SPEF2 genes polymorphisms with semen quality in Russian Holstein bulls (pilot study). *Animals*, 11(10), 2882. https://doi.org/10.3390/ani11102882.

Njubi, D.M., Rege, J.E.O., Thorne, W., Collins-Lusweti E., and Nyambaka, R. 1992. Genetic and environmental variation in reproductive and lactational performance of Jersey cattle in the coastal lowland semi-humid tropics. *Tropical Animal Health and Production*, 24(4), 231-241.

Ojango, J.M. and Pollott, G.E. 2001. Genetic of milk yield and fertility traits in Holstein-Friesian cattle on large-scale Kenyan farms. *Journal Animal Science*., 79, 1742- 1750.

Olsen, H.B., Heringstad, B. and Klemetsdal, G. 2020. Genetic analysis of semen characteristic traits in young Norwegian Red bulls. *Journal of Dairy Science*, 103(1), 545-555. https://doi.org/10.3168/jds.2019-17291.

Orenge, J.S.K., Ilatsia, E.D., Kosgey, I.S. and Kahi, A.K. 2009. Genetic and phenotypic parameters and annual trends for growth and fertility traits of Charolais and Hereford beef cattle breeds in Kenya. *Tropical Animal Health and Production*, 41, 767-774. https://doi.org/10.1007/s11250-008-9250-2.

Osman, M., El-Bayomi, K. and Moawed, S. 2013. Estimation of heritabilities, genetic correlations, phenotypic correlations and genetic trends for production and reproduction traits of Holstein-Friesian dairy cattle using sire model. *Suez Canal Veterinary Medical Journal*, 18(1), 115-128. https://doi.org/10.21608/scvmj.2013.78285.

Otaviano, A.R., Tonhati, H., Sena, J.A.D. and Cerón Muñoz, M.F. 2005. Kappa-casein gene study with molecular markers in female buffaloes (Bubalus bubalis). *Genetics and Molecular Biology*, 28, 237-241. https://doi.org/10.1590/S1415-47572005000200010.

Othman, O.E. and Abdel-Samad, M.F. 2013. RFL polymorphism of three fertility genes in Egyptian buffalo. *Journal of Applied Biological Sciences*, 7(2), 94-101.

Othman, O.E., Zayed, F.A., AM, A.E.G. and El-Rahman, A., 2010. Genetic polymorphism of buffalo μ-CALPAIN gene using PCR-RFLP. *Zagazig Journal of Agricultural Research*, 37(3), 643-654. https://doi.org/10.21608/zjar.2010.317694.

Othman, O.E., Zayed, F.A., El Gawead, A.A. and El-Rahman, M.R., 2011a. Genetic polymorphism of two genes associated with carcass trait in Egyptian buffaloes. *Journal of Genetic Engineering and Biotechnology*, 9(1), 15-20. https://doi.org/10.1016/J.JGEB.2011.05.003.

Othman, O.E., Zayed, F.A., El Gawead, A.A. and El-Rahman, M.R., 2011b. Genetic polymorphism of three genes associated with milk trait in Egyptian buffalo. *Journal of Genetic Engineering and Biotechnology*, 9(2), 97-102. https://doi.org/10.1016/j.jgeb.2011.09.002.

Othman, O.E., Zayed, F.A., El Gawead, A.A. and El-Rahman, M.R., 2012. Genetic polymorphism of three genes (PRL, K-CN and PIT-1) associated with milk traits in Egyptian buffalo. *Genes, Genomes and Genomics*, 6, 97-101. https://doi.org/10.1016/J.JGEB.2011.09.002.

Othman, O.E., Abou-Eisha, A. and El-Din, A.E., 2018. Study on genetic polymorphism of IGFBP-3 gene in Egyptian buffalo. *Annual Research & Review in Biology*, 29(3), 1-7. https://doi.org/10.9734/ARRB/2018/44959.

Özdil, F. and İlhan, F., 2012. DGAT1-exon 8 polymorphism in Anatolian buffalo. *Livestock Science*, 149(1-2), 83-87. https://doi.org/10.1016/j.livsci.2012.06.030.

Özkan Ünal, E., Işık, R. and Soysal, M. I. 2020. Identification of novel single nucleotide polymorphisms in the growth hormone (GH) gene in Anatolian water buffalo (Bubalus bubalis) populations in Turkey. *Brazilian Journal of Animal Science*, 49, e20190260. https://doi.org/10.37496/rbz4920190260.

Özşensoy, Y. 2018. Investigation of PRL-RsaI and HaeIII gene polymorphisms in Anatolian water buffaloes bred by using PCR-RFLP method. *Revista Brasileira de Zootecnia*, 47, e20170166. https://doi.org/10.1590/rbz4720170166.

Páez, M.A.R., Tovar, N.J.R., Mendoza-Sánchez, G., Muñoz, M.F., Molina, B.J.S., Torres, A.F., Gutíerrez, J.A. and Buitrago, J.D. 2021. Sequencing and analysis of the myostatin gene (GDF-8) in Bubalus bubalis young animals to determine the existence of possible mutations expressed in double musculature phenotype. *Journal of Buffalo Science*, 10, 1-5.

Pal, A., Chakravarty, A.K. and Chatterjee, P.N., 2014. Polymorphism of growth hormone gene and its association with seminal and sexual behavioral traits in crossbred cattle. *Theriogenology*, 81(3), 474-480. https://doi.org/10.1016/j.theriogenology.2013.11.002.

Panigrahi, S.K. and Yadav, B.R. 2010. Polymorphism in TNP-1 gene of Murrah buffalo bulls. *African Journal of Biotechnology*, 9(43), 7224-7229. https://doi.org/10.5897/AJB09.165.

Parikh, R.C., Patel, J.V., Patel, A.B., Patel, K.S., Patel, T.B., Patil, R.C., Kansara, J.D., Jakhesara, S.J. and Rank, D.N. 2016. *DGAT1* gene polymorphisms and its association with milk production traits in Mehsana buffalo (Bubalus bubalis). *Buffalo Bulletin*, 35(2), 237-246.

Pauciullo, A., Cosenza, G., Steri, R., Coletta, A., Jemma, L., Feligini, M., Di Berardino, D., Macciotta, N.P. and Ramunno, L. 2012a. An association analysis between OXT genotype and milk yield and flow in Italian Mediterranean river buffalo. *Journal of Dairy Research*, 79(2), 150-156. https://doi.org/10.1017/S0022029911000914.

Pauciullo, A., Cosenza, G., Steri, R., Coletta, A., La Battaglia, A., Di Berardino, D., Macciotta, N.P. and Ramunno, L. 2012b. A single nucleotide polymorphism in the promoter region of river buffalo stearoyl CoA desaturase gene (SCD) is associated with milk yield. *Journal of Dairy Research*, 79(4), 429-435. https://doi.org/10.1017/S0022029912000507.

Pawar, V., Brahmkshtri, B., Pandya, G.M., Janmeda, M., Dangar, N.S., Bayan, J. and Tyagi, K. 2018. Non genetic factors affecting lactation milk yield and estimation of genetic, environmental and phenotypic trends in Surti buffalo. *Indian Journal of Dairy Science*, 71, 491-495.

Peakall, R.O.D. and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.

Peeva, T. and Krastanov, J. 2001. Genetic trend in Bulgarian buffalo population. *Proceedings of the 32nd Biennial Session of ICAR, Bled, Slovenia, 14-19 May, 2001*, 371-374.

Poor, K.H., Babak, M.M.S., Sadeghi, M. and Kianzad, D. 2012. A phenotypic and genetic study of production traits of Iranian buffalloes. *Iranian Journal of Animal Science*, 43 (2), 279-286. https://doi.org/10.22059/ijas.2012.28536.

Ptak, E. and Schaeffer, L.R. 1993. Use of test day yields for genetic evaluation of dairy sires and cows. *Livestock Production Science*, 34(1-2), 23-34. https://doi.org/10.1016/0301-6226(93)90033-E.

Putra, W.P.B., Said, S., Jihaddudin, M.N.F. and Handarini, R. 2020. Single nucleotide polymorphisms (SNPs) in bovine follicle stimulating hormone receptor (bFSHR) gene in Pasundan cattle. *Indian Journal of Animal Sciences*, 90(8), 1195-1197. https://doi.org/10.56093/ijans.v90i8.109316.

Rahbar, R., Aminafshar, M., Abdullahpour, R. and Chamani, M. 2016. Genetic and phenotypic trends of fertility traits for Holstein dairy population in warm and temperate climate. *Journal of Animal*

Behaviour and Biometeorology, 4(2), 43-49. https://doi.org/10.14269/2318-1265/jabb.v4n2p43-49.

Ramadan, S., Saker, A.A. and Shafik, B. 2020. DNA polymorphism of *FSHR* gene and its association with infertility traits in Egyptian buffaloes. *Benha Veterinary Medical Journal*, 39(1), 1-4. https://doi.org/10.21608/bvmj.2020.34139.1221.

Ramadan, S.I., Sakr, A.A. and Shafik, B., 2023. Genetic and non-genetic parameters for productive and reproductive traits of Egyptian buffaloes. *Benha Veterinary Medical Journal*, 43(2), 40-44. https://doi.org/10.21608/bvmj.2022.172187.1607.

Ramesha, K.P., Rao, A., Basavaraju, M., Geetha, G.R., Kataktalware, M.A. and Jeyakumar, S. 2015. Genetic variability of bovine GHR, IGF-1 and IGFBP-3 genes in Indian cattle and buffalo. *South African Journal of Animal Science*, 45(5), 485-493. https://doi.org/10.4314/sajas.v45i5.5.

Ramírez Toro, E.J., Burgos Paz, W.O., Elzo, M.A., Martínez Sarmiento, R.A. and Cerón-Muñoz, M.F. 2020. Genetic parameters and trends for growth traits in Blanco Orejinegro cattle. *Translational Animal Science*, 4(3), 174. https://doi.org/10.1093/tas/txaa174.

Ramos, A.D.A., Malhado, C.H.M., Carneiro, P.L.S., Gonçalves, H.C. and Azevedo, D.M.M.R. 2006. Phenotypic and genetic characterization of the milk yield and calving interval in buffalo of the Murrah breed. *Pesquisa Agropecuária Brasileira*, 41, 1261-1267. https://doi.org/10.1590/S0100-204X2006000800008.

Ranjan, A., Jain, A., Verma, A., Sinha, R., Joshi, P., Gowane, G.R. and Alex, R., 2023. Optimization of test day for milk yield recording and sire evaluation in Murrah buffaloes. *Journal of Animal Breeding and Genetics*, 140(4), 400-412. https://doi.org/10.1111/jbg.12767.

Reen, J.K., Ramesha, K.P., Preeti, S.D. and Rohit, K. 2018. Molecular characterization of luteinizing hormone receptor (LHR) gene in Murrah bulls. *Journal of Entomology and Zoology Studies*, 6(3), 246-250.

Rege, J.E.O. and Mosi , R.O. 1989. Analysis of the Kenyan Friesian breed from 1968 to 1984: genetic and environmental trends and related parameters of milk production. Bulletin of Animal Health Production in Africa, 37, 267.

Rehman, M.S.U., Hassan, F.U., Rehman, Z.U., Ishtiaq, I., Rehman, S.U. and Liu, Q. 2022. Molecular characterization of TGF-beta gene family in buffalo to identify gene duplication and functional mutations. *Genes*, 13(8), 1302. https://doi.org/10.3390/genes13081302.

Rezende, M.P.G.D., Carneiro, P.L.S., Araujo, J.I.M., Araujo, A.C., Campos, B., Moretti, R., Sitorsky, L.G., Fonseca, W.J.L., Bozzi, R. And Malhado, C.H.M. 2020. Heritability and genetic correlations between weight gains in Murrah, Mediterranean, and Jaffarabadi buffaloes raised in Brazil, employing Bayesian inference. *Turkish Journal of Veterinary & Animal Sciences*, 44(2), 337-342. https://doi.org/10.3906/vet-1905-71.

Rolim Filho, S.T., Ribeiro, H.F.L., de Camargo, G.M.F., Cardoso, D.F., Aspilcueta- Borquis, R.R., Tonhati, H., Nunes, K.B., Vale, W.G., Barbosa, E.M. and de Sousa, K.C. 2013. Identification of polymorphisms in the osteopontin gene and their associations with certain semen production traits of water buffaloes in the Brazilian A mazon. *Reproduction in Domestic Animals*, 48(5), 705-709. https://doi.org/10.1111/rda.12144.

Rosati, A. and Van Vleck, L.D. 2002. Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for the Italian river buffalo Bubalus bubalis population. *Livestock Production Science*, 74(2), 185-190. https://doi.org/10.1016/S0301-6226(01)00293-7.

Rushdi, H.E., Saad, M.F., El-Naggar, H.A. and Saeed, A.M. 2017. Association of chromosomal aberrations and semen quality in Egyptian buffalo bulls. *Egyptian Journal of Animal Production*, 54(2), 95-103.

Sahana, G. and Sadana, D.K. 1998. Evaluation of the production performance of Murrah buffaloes. *Indian Journal of Dairy Science*, 51, (2), 115-120.

Sahoo, S.K., Singh, A., Gupta, A.K., Chakravarty, A.K., Singh, M. and Ambhore, G.S. 2014. Estimation of genetic parameters of weekly test-day milk yields and first lactation 305-day milk yield in Murrah buffaloes. *Veterinary World*, 7(12), 1094-1098.

Salem, M.M.I., Amin, A.M.S., Ashour, A.F., Ibrahim, M.M.E.S. and Abo-Ismail, M.K. 2020. Genetic parameters and principal components analysis of breeding value for birth and weaning weight in Egyptian buffalo. *Animal Bioscience*, 34(1), 12-19. https://doi.org/10.5713/ajas.19.0651.

Salem, M.M., Amin, A.M., Ashour, A.F. and El Nagar, A.G. 2023. Estimation of genetic parameters for semen traits in Egyptian buffalo bulls. *Tropical Animal Health and Production*, 55(4), 264. https://doi.org/10.1007/s11250-023-03680-7.

Sallam, E.A., Mohammed, L., Darwish, S., Hegazy, M., El-Naby, A.S. and Azzam, A. 2022. Detection of *FSHR* gene variants associated with sperm motility in Egyptian buffalo bulls. *Benha Veterinary Medical Journal*, 42(2), 191-193. https://doi.org/10.21608/bvmj.2022.143994.1532.

Sanad, S.S. and Gharib, M.G. 2017. Estimation of genetic parameters for some productive and reproductive traits with six different models for Friesian cattle raised in Egypt. *Egyptian Journal of Agricultural Research*, 95(3), 1311-1323. https://doi.org/10.21608/ejar.2017.150285.

Sang, L., Du, Q.Z., Yang, W.C., Tang, K.Q., Yu, J.N., Hua, G.H., Zhang, X.X. and Yang, L.G. 2011. Polymorphisms in follicle stimulation hormone receptor, inhibin alpha, inhibin bata A, and prolactin genes, and their association with sperm quality in Chinese Holstein bulls. *Animal Reproduction Science*, 126(3-4), 151-156. https://doi.org/10.1016/j.anireprosci.2010.02.004.

Sanghuayphrai, N., Nakavisut, S., Dongpaletum, C., Phothikanit, G. and Supanun, S. 2013. Genetic parameters and trends for weaning weight and calving interval of department of livestock development Swamp buffalo**.** *Buffalo Bulletin*,.32, 717-722.

Schaeffer, L.R., Jamrozik, J., Kistemaker, G.J., Van Doormaal, J. 2000 Experience with a Test-Day Model. *Journal Dairy Science*, 83, 1135-1144.

Seidel, G.E. 2009. Brief introduction to whole-genome selection in cattle using single nucleotide polymorphisms. *Reproduction, Fertility and Development*, 22(1), 138-144. https://doi.org/ 10.1071/RD09220.

Sejrsen, K., Purup, S., Vestergaard, M., Weber, M.S. and Knight, C.H., 1999. Growth hormone and mammary development. *Domestic Animal Endocrinology*, 17(2-3), 117-129. https://doi.org/10.1016/S0739-7240(99)00029-6.

Sellier, P., 2000. Genetically caused retarded growth in animals. *Domestic Animal Endocrinology*, 19(2), 105-119. https://doi.org/10.1016/s0739-7240(00)00071-0.

Seno, L.O., Cardoso, V.L., El Faro, L., Sesana, R.C., Aspilcueta-Borquis, R.R., De Camargo, G.M.F. and Tonhati, H. 2010. Genetic parameters for milk yield, age at first calving and interval between first and second calving in milk Murrah buffaloes. *Livestock Research for Rural Development*, 22(2), 38.

Sesana, R.C., Bignardi, A.B., Borquis, R.R.A., El Faro, L., Baldi, F., Albuquerque, L.G.D. and Tonhati, H., 2010. Random regression models to estimate genetic parameters for test- day milk yield in Brazilian Murrah buffaloes. *Journal of Animal Breeding and Genetics*, 127(5), 369-376. https://doi.org/10.1111/j.1439-0388.2010.00857.x.

Setyorini, Y.W., Kurnianto, E., Sutopo, S. and Sutiyono, S., 2023. Identification polymorphism of LHR and *FSHR* genes in Indonesian Holstein dairy cattle associated with productive and reproductive traits.

Biodiversitas Journal of Biological Diversity, 24(5). https://doi.org/10.13057/biodiv/d240544.

Shadma Fatima, S.F., Bhatt, S.M., Bhong, C.D., Rank, D.N. and Joshi, C.G. 2009. Genetic polymorphism study of IGF-I gene in buffaloes of Gujarat. *Buffalo Bulletin*, 28(3),159-164.

ShafiK Basant M.N., El-Bayomi, Kh. M., Abo-Salem, M.E.S. and Darwish, S.A. 2017. Environmental factors affecting some productive and reproductive traits in Egyptian buffaloes. *Benha Veterinary Medical Journal*, 32(1), 153-159.

ShafiK Basant M.N., El-Bayomi, Kh.M., Abo-Salem, M.E.S., Darwish, S.A. and Ramadan, S.I. 2017. Detection of SNPs in Exon10 Locus of *FSHR* Gene and Their Association with Some Infertility problems in Egyptian Buffaloes. *Benha Veterinary Medical Journal*, 32(1),160-169.

Shahin, K.A., Abdallah, O.Y., Fooda, T.A. and Mourad, K.A. 2010. Selection indexes for genetic improvement of yearling weight in Egyptian buffaloes. *Archives Animal Breeding*, 53(4), 436-446.

Shalaby, N.A., Oudah, E.Z.M. and El-Sharkawy, Y. 2016. Comparison between some productive and reproductive traits and genetic parameters in the first three lactations in Egyptian buffaloes. *Journal of Animal and Poultry Production*, 7(3), 113-119. https://doi.org/10.21608/jappmu.2016.48518.

Shao, B., Sun, H., Ahmad, M.J., Ghanem, N., Abdel-Shafy, H., Du, C., Deng, T., Mansoor, S., Zhou, Y., Yang, Y. and Zhang, S. 2021. Genetic features of reproductive traits in bovine and buffalo: Lessons from bovine to buffalo. *Frontiers in Genetics*, 12, 617128. https://doi.org/10.3389/fgene.2021.617128.

Sharif-Islam, Md. and Bhuiyan, A.K.F.H., 2024. Phenotypic and genetic trends in growth traits in a nucleus herd of Red Chittagong cattle. *Journal of Dairy Research and Technology*, 6(029), 1-7. https://doi.org/10.24966/DRT-9315/100029.

Sharma, R.C. and Singh, B.P. 1992. Genetic trends in Murrah buffaloes. *Indian Veterinary Journal*, 69 (3), 271-272.

Sihag, S., Rushdi, H.E., Kumar, A., Jangra, A., Hassanane, M.S., Hamdy, A.S. and Chhokar, V. 2023. Polymorphic variants analysis in Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) Gene of Indian and Egyptian buffaloes. *Indian Journal of Animal Research*, 57(11), 1474-1479. https://doi.org/10.18805/IJAR.B-5182.

Silva, C.S., Silva Filho, E., Matos, A.S., Schierholt, A.S., Costa, M.R., Marques, L.C., Costa, J.S., Sales, R.L., Figueiró, M.R. and Marques, J.R.F. 2016. Polymorphisms in the *DGAT1* gene in buffaloes (Bubalus bubalis) in the Amazon.*Genetics and Molecular Research*, 15(3), 1-7. https://doi.org/10.4238/gmr.15038720.

Silva, C.S., da Silva Filho, E., da Silva, L.K.N., Ferraz, R.S., de Sousa Matos, A., Marques, L.C., Sales, R.L., de Araújo Guimarães, D.A. and Marques, J.R.F. 2021. Polymorphisms in the Leptin and SCD genes in buffaloes of the Amazon. *Research, Society and Development*, 10(13), e233101321222. https://doi.org/ https://doi.org/10.33448/rsd-v10i13.21222.

Simoni, M., Gromoll, J. and Nieschlag, E. 1997. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocrine Reviews*, 18(6), 739-773. https://doi.org/10.1210/edrv.18.6.0320.

Singh, M., Singh, A., Gupta, A.K., Dash, S.K., Gupta, A., Sahoo, S.K., Dash, S. and Shivahre, P.R. 2015. Comparative evaluation of different lactation curve models in prediction of monthly test-day milk yields in Murrah buffaloes. *Journal of Animal Research*, 5(1), 189-193. https://doi.org/10.5958/2277-940X.2015.00030.

Singh, M., Singh, A., Gupta, A.K., Dash, S.K., Shivahre, P.R., Sahoo, S.K. and Ambhore, G.S. 2016. Genetic parameters of 305 days and

monthly test-day milk yields in Murrah buffaloes. *Indian Journal of Animal Sciences*, 86(1), 55-60.

Singh, A.P., Ramesha, K.P., Mir, M.A., Arya, A. and Isloor, S. 2020. Variation in lactoferrin gene affects milk lactoferrin content and somatic cell count in Murrah buffaloes. *Indian Journal of Animal Research*, 54(3), 265-274. https://doi.org/10.18805/ijar.B-3773.

Sinha, Y.N., 1995. Structural variants of prolactin: occurrence and physiological significance. *Endocrine Reviews*, 16(3), 354-369. https://doi.org/10.1210/edrv-16-3-354.

Sosa, A.S.A., Mahmoud, K.G.M., Eldebaky, H.A.A., Kandiel, M., Abou El-Roos, M. and Nawito, M. 2015. Genotyping of follicle stimulating hormone receptor gene in fertile and infertile buffalo. *Global Veterinaria*, 15, 163-168. https://doi.org/10.5829/idosi.gv.2015.15.02.96166.

Sosa, A.S.A., Mahmoud, K.G.M., Kandiel, M.M.M., Eldebaky, H.A.A., Nawito, M.F. and Abou El-Roos, M.E.A., 2016. Genetic polymorphism of luteinizing hormone receptor gene in relation to fertility of Egyptian buffalo. *Biotechnology*: An Indian Journal, 12.

Strabel, T., Szyda, J., Ptak, E. and Jamrozik, J. 2005. Comparison of random regression test-day models for Polish Black and White cattle. *Journal of Dairy Science*, 88(10), 3688-3699. https://doi.org/10.3168/jds.S0022-0302(05)73055-1.

Suhail, S.M., Qureshi, M.S., Sarzamin Khan, S.K., Ihsanullah, I. and Durrani, F.R. 2009. Inheritance of economic traits of dairy buffaloes in Pakistan. *Sarhad Jurnal Agriculture*, 25(1), 87-93.

Sulabh, S., Verma, A., Gupta, I.D. and Kumar, B. 2018. Novel SNPs in *DGAT1* gene and their association with milk traits in Murrah buffaloes. Indian *Journal Dairy Science*, 71(2), 187-192.

Swalve, H.H. 1995. The effect of test day models on the estimation of genetic parameters and breeding values for dairy yield traits. *Journal of Dairy Science*, 78(4), 929-938. https://doi.org/10.3168/jds.S0022-0302(95)76708-X.

Tăbăran, A., Balteanu, V.A., Gal, E., Pusta, D., Mihaiu, R., Dan, S.D., Tăbăran, A.F. and Mihaiu, M. 2015. Influence of *DGAT1* K232A polymorphism on milk fat percentage and fatty acid profiles in Romanian Holstein cattle. *Animal Biotechnology*, 26(2), 105-111. https://doi.org/10.1080/10495398.2014.933740.

Tanpure, T., Dubey, P.K., Singh, K.P., Kathiravan, P., Mishra, B.P., Niranjan, S.K. and Kataria, R.S. 2012. PCR-SSCP analysis of leptin gene and its association with milk production traits in river buffalo (Bubalus bubalis). *Tropical Animal Health and Production*, 44, 1587-1592. https://doi.org/10.1007/s11250-012-0111-7.

Tawah, C.L., Rege, J.E.O., Mbah, D.A. and Oumate, H. 1994. Genetic evaluation of birth and weaning weight of Gudali and two-breed synthetic Wakwa beef cattle populations under selection in Cameroon: Genetic and phenotypic trends. *Animal Science*, 58(1), 25-34. https://doi.org/10.1017/S0003356100007042.

Thiruvenkadan, A.K., Panneerselvam, S. and Rajendran, R. 2009. Non-genetic and genetic factors influencing growth performance in Murrah Buffalos. *South African Journal of Animal Science*, 39 (Sup-1), 102-106.

Thiruvenkadan, A.K., Panneerselvam, S., Rajendran, R. and Murali, N. 2010. Analysis on the productive and reproductive traits of Murrah buffalo cows maintained in the coastal region of India. *Applied Animal Husbandry and Rural Development*, 3(1), 1-5.

Tonhati, H., Cerón-Muñoz, M.F., Oliveira, J.A.D., El Faro, L., Lima, A.L.F. and Albuquerque, L.G.D. 2008. Test-day milk yield as a selection criterion for dairy buffaloes (Bubalus bubalis Artiodactyla, Bovidae). *Genetics and Molecular Biology*, 31, 674-679. https://doi.org/10.1590/S1415-47572008000400012.

Trakovická, A., Moravčíková, N. and Minarovič, T. 2013. PCR-RFLP analyses for studying the diversity of *GH* and Pit-1 genes in Slovak Simmental cattle. Scientific Papers: *Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehniesi Biotehnologii*, 46(2).

Vale, W.G., Purohit, G.N., Miyasaki, M.Y. and Gaur, M. 2014. Semen characteristics and artificial insemination in the buffalo. *Bubaline Theriogenology. G. N. Purohit (Ed.)*, 524-559.

Wagdy, M., Abdelrazek, E., Zaghloul, A., Sakr, M.A., Elweza, A. and Elbaz, H., 2023. Genetic polymorphism of CYP19 and FSHR genes and their association with ovarian inactivity and silent heat in Egyptian buffaloes (Bubalus bubalis). Journal of Current Veterinary Research, 5(1), 231-240.

Wang, G., Hao, L., Cheng, Y., Li, S., Zhang, Y., Lv, C., Wei, W., Huang, S., Shi, H., Dong, L. and Zhang, Y. 2017. Effects of GnRHR polymorphisms on sperm quality in Chinese water buffalo. *Animal Reproduction Science*, 186,37-43. https://doi.org/10.1016/j.anireprosci.2017.09.001.

Wang, S., Zhang, Y., Cheng, Y., Lu, G., Yang, R., Geng, H., Wang, C., Li, H., Feng, T., Liu, S. and Hao, L. 2020. Association of SNPs in GnRH gene with sperm quality traits of Chinese water buffalo. *Reproduction in Domestic Animals*, 55(3), 384-392. https://doi.org/10.1111/rda.13634.

Xiong, Z., Zhang, H., Huang, B., Liu, Q., Wang, Y., Shi, D. and Li, X. 2018. Expression pattern of prohibitin, capping actin protein of muscle Z-line beta subunit and tektin-2 gene in Murrah buffalo sperm and its relationship with sperm motility. *Asian-Australasian Journal of Animal Sciences*, 31(11), 1729-1737. https://doi.org/10.5713/ajas.18.0025.

Yang, W., Kang, X., Yang, Q., Lin, Y. and Fang, M. 2013. Review on the development of genotyping methods for assessing farm animal diversity. *Journal of Animal Science and Biotechnology*, 4 (2), 1-6. https://doi.org/:10.1186/2049-1891-4-2.

Yin, H., Fang, L., Qin, C. and Zhang, S. 2019. Estimation of the genetic parameters for semen traits in Chinese Holstein bulls. *BMC Genetics*, 20, 1-5. https://doi.org/10.1186/s12863-019-0752-x.

Yuan, J., Zhou, J., Deng, X., Hu, X. and Li, N. 2007. Molecular cloning and single nucleotide polymorphism detection of buffalo *DGAT1* gene. *Biochemical Genetics*, 45, 611-621. https://doi.org/10.1007/s10528-007-9100-3.

Zahed, S.M., Badr, A.A. and Khattab, A.S. 2020. Genetic and phenotypic association between productive and reproductive traits in Friesian cows in Egypt. *Journal of Animal and Poultry Production*, Mansoura University, 11(3), 83-88. https://doi.org/10.21608/jappmu.2020.87123.

Zetouni, L., De Camargo, G.M.F., da Silva Fonseca, P.D., Gil, F.M.M., Lugo, N.A.H., Aspilcueta-Borquis, R.R., Cervini, M. and Tonhati, H., 2013. Effects of a single nucleotide polymorphism in the leptin gene on the productive traits of dairy buffaloes (Bubalus bubalis). *Molecular Biology Reports*, 40, 5159-5163. https://doi.org/10.1007/s11033-013-2618-z.

Zetouni, L., de Camargo, G.M.F., da Silva Fonseca, P.D., Cardoso, D.F., Gil, F.M.M., Hurtado-Lugo, N.A., Aspilcueta-Borquis, R.R., Cervini, M. and Tonhati, H. 2014. Polymorphisms in the MTRN1A gene and their effects on the productive and reproductive traits in buffaloes. *Tropical animal health and production*, 46, 337-340. https://doi.org/10.1007/s11250-013-0493-1.

الملخص العربي

إشتملت الأهداف الرئيسة لهذه الدراسة على: 1- تقييم بعض صفات الإدرار، التناسل، السائل المنوي والنمو في بعض قطعان الجاموس المصري وذلك عن طريق تقدير مكونات التباين والمكافئ الوراثي بإستخدام طريقة الجبس Bayesian Gibbs Sampling Algorithm بتطبيق نموذج الحيوان وحيد الصفة ونموذج الإنحدار العشوائي Random Regression Model (RRM)، 2- التنبؤ بالقيم التربوية (PBVs) ورسم الإتجاهات الوراثية والمظهرية لهذه الصفات بإستخدام برنامج BLUPF90، 3- توصيف بعض الجينات المرشحة *FSHR، DGAT1، PRL، GH* في الجاموس المصري على أساس التنوع الوراثي الجزيئي على مستوى النيوكليوتيدة الواحدة SNPs، 4- إستخدام تقنية PCR-RFLP في تحديد النمط الجيني للتراكيب الوراثية الموجودة في المناطق المحفزة لهذه الجينات، 5- كشف الإرتباطات الجزيئية للتراكيب الوراثية للجينات *PRL، FSHR، GH* المرشحة مع صفات إنتاج اللبن ومكوناته، التناسل، السائل المنوي ووزن الجسم في الجاموس المصري بإستخدام متوسطات المربعات الصغرى المعممة Generalized Least Square means (GLSM).

بالنسبة للتحليلات الوراثية الكمية، تم إستخدام أربعة مجموعات من البيانات لصفات الإدرار، التناسل، السائل المنوي وصفات وزن الجسم. تم تسجيل عدد 7345 سجلاً منسباً لبيانات يوم الإختبار لكل شهر من الإدرار (TD) لصفات محصول اللبن ،محصول الدهن ،محصول البروتين وعدد الخلايا الجسمية باللبن لعدد 686 جاموسة ناتجة من 83 أب ، 423 أم خلال 21 عام من 2003 إلى 2023 في ثلاث قطعان تجريبية للجاموس المصري (النطاف الجديد، النطاف القديم والجميزة)، كما تم جمع عدد 7279 سجلاً لصفات التناسل (العمر عند أول ولادة، عدد الأيام المفتوحة والفترة بين ولادتين) خلال 22 عاماً من 2002 إلى 2023 لعدد 1951 جاموسة ناتجة من 155 أب ، 1179 أم في ستة قطعان تجريبية (النطاف الجديد، النطاف القديم، النوبارية، السرو، الجميزة وسدس) ، كما تم جمع بيانات 5178 عينة من السائل المنوي من 111 طلوقة جاموسي ناتجة من 34 أب و92 أم خلال 10 أعوام من 2013 إلى 2022 في قطيع المركز الدولي للتدريب على رعاية الحيوان بسخا (IMTC) وقطيع محلة موسى (MM) بمحافظة كفر الشيخ. تم جمع البيانات عن وزن الجسم عند الولادة والفطام لعدد 8229 من عجول الجاموس ناتجة من 277 أب، 2175 أم خلال 22 عاماً من 2003 إلى 2024 في ستة قطعان تجريبية (النطاف الجديد، النطاف

القديم، النوبارية، السرو، الجميزة وسدس) التابعة لمعهد بحوث الإنتاج الحيواني (APRI)، مركز البحوث الزراعية، وزارة الزراعة وإستصلاح الأراضي، مصر.

بالنسبة للتحليلات الوراثية الجزيئية، تم جمع عينات دم من 286 حيواناً (200 أنثي و86 ذكر) من حيوانات الجاموس تم إختيارهم عشوائياً من ثلاثة قطعان تجريبية (النطاف الجديد، النطاف القديم والجميزة) وذلك لتحديد أنماط التراكيب الوراثية بإستخدام تقنية PCR–RFLP ودراسة الإرتباطات الوراثية الجزيئية للجينات *GH، FSHR، PRL* مع صفات الإدرار، التناسل، السائل المنوي والنمو.

خلصت نتائج هذه الدراسة على ما يلي:

<u>التحليلات الوراثية الكمية لصفات الإدرار</u>

كانت قيم المكافئ الوراثي المحسوبة من نموذج الحيوان وحيد الصفة غالباً متوسطة وتراوحت بين 0.05 إلى 0.40 لمحصول اللبن عند يوم الإختبار، 0.05 إلى 0.45 لمحصول الدهن عند يوم الإختبار، 0.06 إلى 0.44 لمحصول البروتين عند يوم الإختبار، 0.03 إلى 0.39 لعدد الخلايا الجسمية باللبن في حين كانت قيم المكافئ الوراثي المقدرة بإستخدام نموذج الإنحدار العشوائي منخفضة غالباً في بداية الإدرار ثم ترتفع تدريجياً لتصل إلى أعلى قيمة ثم تنخفض تدريجياً إلى أدنى قيمة قرب نهاية الإدرار وتراوحت القيم بين 0.04 إلى 0.25 لمحصول اللبن عند يوم الإختبار،0.05 إلى 0.18 لمحصول الدهن عند يوم الإختبار، 0.03 إلى 0.23 لمحصول البروتين عند يوم الإختبار، 0.07 إلى 0.57 لعدد الخلايا الجسمية باللبن عند يوم الإختبار. كانت القيم التربوية المتنبأ بها متوسطة إلى عالية وتراوحت بين –2.01 إلى 3.4 كجم لمحصول اللبن عند يوم الإختبار، –358 إلى 521 جرام لمحصول الدهن عند يوم الإختبار، –53 إلى 95 جرام لمحصول البروتين عند يوم الاختبار، –0.183 إلى 0.313 $\log^{10}$ لعدد الخلايا الجسمية باللبن عند يوم الإختبار. إرتفعت قيم الإتجاهات الوراثية إرتفاعا ملحوظاً وإيجابياً من –4.63 ليصبح 1.61 كجم لمحصول اللبن عند يوم الإختبار، من –5.0 ليصبح 495 جراماً لمحصول الدهن عند يوم الإختبار، من –26 ليصبح 280 جراماً لمحصول البروتين عند يوم الإختبار، إلى جانب إنخفاض إيجابي مرغوب في قيم الإتجاهات الوراثية لعدد الخلايا الجسمية باللبن عند يوم الإختبار من1.37 ليصبح 1.19 $\log^{10}$ مع تقدم سنة الإدرار. على العكس من ذلك إنخفضت الإتجاهات المظهرية لصفات الإدرار بشكل غير مرغوب وتراوحت قيمته من 7.49 ليصبح 5.69 كجم لمحصول اللبن عند يوم الإختبار، من 510 ليصبح 360 جراماً لمحصول الدهن عند يوم الإختبار، من 284 ليصبح 223 جراماً لمحصول البروتين عند يوم الإختبار مع زيادة غير مرغوبة في عدد الخلايا الجسمية باللبن عند يوم الإختبار بمقدار من 1.62 لتصبح 2.43 $\log^{10}$ مع تقدم سنة الإدرار.

التحليلات الوراثية الكمية لصفات التناسل

كانت قيم المكافئ الوراثي للصفات التناسلية لإناث الجاموس المقدرة بإستخدام نموذج الحيوان وحيد الصفة منخفضة وبلغت 0.10 للعمر عند أول ولادة ، 0.02 لعدد الأيام المفتوحة ، 0.02 للفترة بين ولادتين. وكان المدى في القيم التربوية المتنبأ بها متوسطا أو مرتفعا وتراوحت قيمته بين –8.24 إلى 10.84 شهراً للعمر عند أول ولادة ، –124.7 إلى 123.9 يوماً لعدد الأيام المفتوحة ، –141.8 إلى 132.5 يوماً للفترة بين ولادتين، وإنخفضت الإتجاهات الوراثية إيجابياً من 0.24 ليصبح –0.14 شهراً للعمر عند أول ولادة، من 5.5 ليصبح 2.9 يوماً لعدد الأيام المفتوحة، من 6.9 ليصبح 3.6 يوماً للفترة بين ولادتين. وقد لوحظ مدى واسع في قيم الإتجاهات المظهرية لصفات التناسل من 36.57 ليصبح 36.52 شهراً للعمر عند أول ولادة، من 127 ليصبح 71 يوماً لعدد الأيام المفتوحة، من 416 ليصبح 354 يوماً للفترة بين ولادتين.

التحليلات الوراثية الكمية لصفات السائل المنوي

كانت قيم المكافئ الوراثي لصفات السائل المنوي لطلائق الجاموس المقدرة بإستخدام نموذج الحيوان وحيد الصفة متوسطة وبلغت 0.17، 0.28 ، 0.27، 0.27 ، 0.23 لحجم القذفة المنوية وحركة الحيوانات المنوية ونسبة الحيوانات الحية ونسبة الحيوانات المنوية الشاذة وتركيز الحيوانات المنوية علي الترتيب. أما المدى في القيم التربوية المحتملة فكانت متوسطة أو مرتفعة حيث تراوحت بين –0.63 إلى 0.42 ملي لحجم القذفة المنوية، –27.3 إلى 85.0 % لحركة الحيوانات المنوية، –27.3 إلى 81.8 % لنسبة الحيوانات المنوية الحية ، –3.7 إلى 24.8 % لنسبة الحيوانات المنوية الشاذة ، –1.2 إلى 2.5 x 10^9 لتركيز الحيوانات المنوية . في حين إرتفعت قيم الإتجاهات الوراثية لصفات السائل المنوى بشكل مرغوب من 1.99 كجم ليصبح 2.3 ملي لحجم القذفة المنوية، من 36.8 % ليصبح 47.8 % لحركة الحيوانات المنوية، من 35.6 % ليصبح 47.8 % لنسبة الحيوانات المنوية الحية، من 2.3 % ليصبح 5.9% لنسبة الحيوانات المنوية الشاذة، من 0.39 إلى 1.24 x 10^9 لتركيز الحيوانات المنوية. إنخفضت الإتجاهات المظهرية من 4.1 ملي ليصبح 3.1 ملي لحجم القذفة المنوية ومن 68.2 % ليصبح 57.1 % لحركة الحيوانات المنوية ومن 67.4 % ليصبح 56.2 % لنسبة الحيوانات المنوية الحية بينما إزدادت الاتجاهات المظهرية من 3.1 % ليصبح 8.1 % لنسبة الحيوانات المنوية الشاذة ومن 0.6 ليصبح 1.2 x10^9 لتركيز الحيوانات المنوية.

التحليلات الوراثية الكمية لصفات النمو

كانت قيم المكافئ الوراثي لوزن الجسم عند الميلاد، وزن الجسم عند الفطام ومعدل الزيادة اليومية في الوزن من الميلاد حتى الفطام المقدرة بإستخدام نموذج الحيوان وحيد الصفة متوسطة أو مرتفعة غالباً وبلغت 0.26، 0.50 ، 0.55 علي الترتيب. تراوح المدى في القيم التربوية المحتملة بين −4.2 إلى 3.5 كجم للوزن عند الميلاد، −42.4 إلى 44.2 كجم للوزن عند الفطام، −0.44 إلى 0.52 كجم لمعدل الزيادة اليومية في الوزن. وقد زادت الإتجاهات الوراثية لصفات وزن الجسم ومعدل الزيادة اليومية في الوزن زيادة طفيفة حيث تراوحت من 1.6 كجم ليصبح 1.8 كجم للوزن عند الميلاد، من −0.519 كجم ليصبح 1.6 كجم للوزن عند الفطام، من −24 جرام ليصبح 18 جراماً لمعدل الزيادة اليومية في الوزن. بينما إنخفضت الإتجاهات المظهرية لصفات وزن الجسم ومعدل الزيادة اليومية في الوزن إنخفاضا طفيفا من 36.6 كجم ليصبح 32.9 كجم للوزن عند الميلاد، من 94.55 كجم ليصبح 94.15 كجم للوزن عند الفطام، من 628 جرام ليصبح 582 جراماً لمعدل الزيادة اليومية في الوزن.

التنوع الجزيئي للجينات *GH*، *FSHR* ، *DGAT1* ، *PRL* في قطعان الجاموس المختلفة

بالنسبة للأوزان الجزيئية لجين *PRL*، تم هضم القطعة غير المهضومة المتضاعفة بحجم 678 زوج من القواعد بإستخدام إنزيم القطع *Xba*I ومن ثم أنتجت التركيب الوراثي AA على هيئة قطعة واحدة (678 زوج من القواعد)، والتركيب الوراثي GG على هيئة قطعتين (678 ، 447 زوج من القواعد). سجل التركيب الوراثي AA أعلى تكرارت للتراكيب الوراثية (0.851) بينما سجل التركيب الوراثي GG قيما منخفضة (0.149). كما أن تكرار الأليل A أعلى من تكرار الأليل G (0.851 مقابل 0.149). بمقارنة قطيع النطاف الجديد بقطيع النطاف القديم، كانت تكرارات التراكيب الوراثية للجين البرولاكتين متقاربة (0.900 مقابل 0.845 للتركيب AA 0.100 مقابل 0.155 للتركيب الوراثي GG). وبدراسة العدد الفعال للأليلات كمؤشر للتنوع الوراثي، أظهرت النتائج أن الإختلافات في العدد الفعال بين قطيعي النطاف الجديد والنطاف القديم كانت معنوية (1.220 مقابل 1.355). كانت قيم محتوى المعلومات للتنوع الجزيئي (*PIC*) منخفظة في قطيع النطاف الجديد حيث كانت قيمتها 0.157 ومتوسطة 0.223 في قطيع النطاف القديم، ومتوسطة في كلا القطيعين مع بعضهما (0.211) . كانت قيم مربع كاي للتراكيب الوراثية للجين البرولاكتين عالية المعنوية في قطيعي النطاف الجديد والنطاف القديم ومن ثم كانت العشائر في حالة عدم إتزان لهذا الجين. كانت نسب التراكيب الوراثية الخليطة المتوقعة للجين البرولاكتين متوسطة بقيم 0.180 في قطيع النطاف القديم، 0.262 في قطيع النطاف الجديد، 0.253 في كلا القطيعين مع بعضهما.

بالنسبة لجين *DGAT1*، تم الكشف عن تركيب وراثي واحد CC بتكرار 1.0 للأليل C وصفر للأليل T. وكان طول القطعة المتضاعفة المهضومة بإستخدام إنزيم القطع *AluI* هو 411 زوج من القواعد، كما تم الكشف عن تركيب وراثي واحد CC يتكون من ثلاث قطع بأطوال 176 ،167، 68 زوج من القواعد.

بالنسبة للأوزان الجزيئية لجين *FSHR*، تم هضم القطعة غير المهضومة المتضاعفة بحجم 306 زوج من القواعد بإستخدام إنزيم القطع *AluI* ومن ثم أنتجت ثلاث تراكيب وراثية (GG, GC, CC)، وكانت نواتج تفاعل إنزيم البلمرة المتسلسل عبارة عن قطعة واحدة للتركيب الوراثي GG (306 زوج من القواعد) ، قطعتين في التركيب الوراثي CC (243 ، 63 زوج من القواعد) ، وثلاث قطع للتركيب الوراثي GC (306 ، 243، 63 زوج من القواعد). بلغت تكرارات التراكيب الوراثية في ذكور وإناث الجاموس 0.41 للتركيب الوراثي GC، 0.21 للتركيب الوراثي GG، 0.38 للتركيب الوراثي CC، أي أن تكرار الأليل C (0.592) كان أعلى من تكرار الأليل G (0.408). كذلك كانت تكرارات التراكيب الوراثية GG, GC, CC للجين *FSHR* هي 0.35، 0.51، 0.34 لإناث الجاموس، وكانت 0.28، 0.28، 0.44 لذكور الجاموس. كذلك كانت تكرارات التراكيب الوراثية GG, GC, CC للجين *FSHR* في قطعان النطاف الجديد، النطاف القديم والجميزة مختلفة كثيراً (0.212 في قطيع النطاف الجديد ، 0.113 في قطيع النطاف القديم ، 0.095 في قطيع الجميزة للتركيب الواثي GG مقابل 0.515 ، 0.500 ، 0.534 للتركيب الوراثي GC ومقابل 0.272 في قطيع النطاف الجديد، 0.386 في قطيع النطاف القديم، 0.381 في قطيع الجميزة للتركيب الواثي CC). كانت تكرارات الأليل C أعلى من تكرارات الأليل G بقيم 0.530 ، 0.636 ، 0.643 للأليل C مقابل 0.470 ، 0.364 ، 0.357 للأليل G في قطعان النطاف الجديد والنطاف القديم والجميزة على الترتيب. كانت الفروق في العدد الفعال للأليلات للجين *FSHR* معنوية بين القطعان المختلفة وكانت 1.993، 1.862، 1.862 في قطعان النطاف الجديد والنطاف القديم والجميزة على التوالي. كانت قيم محتوى المعلومات للتنوع الجزيئي (*PIC*) متوسطة وكانت قيمتها 0.531 في قطيع النطاف الجديد، 0.653 في قطيع النطاف القديم، 0.662 في قطيع الجميزة. كانت نسبة التراكيب الوراثية الخليطة المتوقعة للجين *FSHR* عالية بقيم 0.498 في قطيع النطاف الجديد، 0.463 في قطيع النطاف القديم، 0.459 في قطيع الجميزة، 0.479 في إناث الجاموس، 0.488 في ذكور الجاموس، بينما كانت نسبة التراكيب الوراثية الخليطة المشاهدة 0.515 في قطيع النطاف الجديد، 0.500 في قطيع النطاف القديم، 0.524 في قطيع الجميزة 0.510 في إناث الجاموس، 0.282 في ذكور الجاموس.

بالنسبة لجين *GH*، تم هضم القطعة غير المهضومة المتضاعفة بحجم 211 زوج من القواعد بإستخدام إنزيم القطع *AluI* ومن ثم أنتجت تركيبين وراثيين هما CC ، TC. وكانت نواتج تفاعل إنزيم البلمرة المتسلسل عبارة عن قطعتين للتركيب الوراثي CC (211 ، 159 زوج من القواعد) ، وثلاث قطع للتركيب الوراثي TC (211 ، 159، 52 زوج من القواعد). سجل التركيب الوراثي CC للجين هرمون النمو أعلى تكرارت للتراكيب الوراثية (0.608 في قطيع النطاف الجديد، 0.505 في قطيع النطاف القديم، 0.500 في قطيع الجميزة) بينما سجل التركيب الوراثي TC قيم (0.392 في قطيع النطاف الجديد، 0.495 في قطيع النطاف القديم، 0.500 في قطيع الجميزة). بالنسبة لجميع القطعان، كانت تكرارات التراكيب الوراثية للتركيب الوراثي CC هي 0.68 لإناث الجاموس، 0.30 للذكور، 0.52 في كلا الجنسين، بينما كانت TC هي 0.32 لإناث الجاموس، 0.70 للذكور، 0.48 في كلا الجنسين. سجلت التكرارات للأليل C (0.804 في قطيع النطاف الجديد، 0.753 في قطيع النطاف القديم، 0.750 في قطيع الجميزة) قيماً أعلى من الأليل T (0.196 في قطيع النطاف الجديد، 0.247 في قطيع النطاف القديم، 0.250 في قطيع الجميزة). وبدراسة العدد الفعال للأليلات بين الثلاث قطعان، أظهرت الإختلافات في العدد الفعال إختلافاً معنوياً. في جميع القطعان حيث سجلت ذكور الجاموس أعلى القيم للعدد الفعال للأليلات (1.839)، بينما سجلت إناث الجاموس أقل قيم للعدد الفعال للأليلات (1.368). كانت الفروق في قيم مربع كاي للتراكيب الوراثية للجين هومون النمو غير معنوية بين الذكور والإناث، مما يشير إلى أن تلك العشائر كانت في حالة إتزان هاردي–فاينبرج. في جميع القطعان، كانت نسب التراكيب الوراثية الخليطة للجين هرمون النمو متوسطة أو مرتفعة وتراوحت بين 0.320 إلى 0.704 لنسب التراكيب الوراثية الخليطة المتوقعة، من 0.269 إلى 0.456 لنسب التراكيب الوراثية الخليطة المشاهدة.

<u>الإرتباطات الجزيئية بين التراكيب الوراثية للجينات *GH* ، *FSHR* ، *PRL* وصفات الإدرار</u>

كانت متوسطات المربعات الصغرى المعممة المحسوبة بإستخدام برنامج *PEST* لصفات الإدرار للتراكيب الوراثية AA ،GG لجين *PRL* في قطيع النطاف الجديد والنطاف القديم معنوية وفي صالح التركيب الوراثي AA .في جميع القطعان، سجل التركيب الوراثي AA أعلى المتوسطات المعممة (6.0 كجم لمحصول اللبن عند يوم الإختبار، 390 جرام لمحصول الدهن عند يوم الإختبار،290 جرام لمحصول البروتين عند يوم الإختبار، 2.47 $\log^{10}$ لعدد الخلايا الجسمية باللبن عند يوم الإختبار مقابل 5.3 كجم ، 340 جرام ، 220 جرام، 2.50 log10 علي الترتيب). كانت المتوسطات المعممة لصفات الادرار في قطيع النطاف الجديد معنوية وفي صالح التركيب الوراثي AA مقارنة بالتركيبب الواثي GG (5.9 مقابل 5.5 كجم لصفات لمحصول اللبن عند يوم

الإختبار، 360 مقابل 310 جرام لمحصول الدهن عند يوم الإختبار، 260 مقابل 220 جرام لمحصول البروتين عند يوم الإختبار، 2.38 مقابل 2.52 $\log^{10}$ لعدد الخلايا الجسمية باللبن عند يوم الإختبار)، وكانت متوسطات المربعات الصغرى المعممة في قطيع النطاف القديم (5.97 مقابل 5.43 كجم، 390 مقابل 350 جرام ،290 مقابل 230 جرام ، 2.41 مقابل 2.49 $\log^{10}$).

كانت المتوسط المعممة لصفات االادرار بين التراكيب الوراثية GG ،GC، CC للجين *FSHR* في مختلف القطعان معنوية وفي صالح التركيب الوراثي C .حيث كانت المتوسطات المعممة في الغالب لصالح التركيب الوراثي CC مقارنة بالتراكيب الوراثية GG ،GC في قطيع النطاف الجديد (6.8 مقابل 5.7 ، 6.0 كجم لمحصول اللبن عند يوم الإختبار، 480 مقابل 380 ، 410 جرام لمحصول الدهن عند يوم الإختبار، 280 مقابل 220 ، 250 جرام لمحصول البروتين عند يوم الإختبار، 2.41 مقابل 2.43 ، 2.49 $\log^{10}$ لعدد الخلايا الجسمية باللبن عند يوم الإختبار) .بينما كانت المتوسطات المعممة في قطيع النطاف القديم (6.8 مقابل 5.4 ، 5.5 كجم ، 390 مقابل 340 ، 320 جرام ، 290 مقابل 225 ، 230 جرام ،2.41 ، 2.45 ، 2.49 $\log^{10}$).

تم الكشف عن تركيبين وراثيين هما TC، CC للجين *GH* لصفات الإدرار في قطيع النطاف الجديد والنطاف القديم ، حيث كانت المتوسطات المعممة معنوية وفي صالح التركيب الوراثي TC . وكانت متوسطات المربعات الصغرى المعممة للتركيب الوراثي TC عالية المعنوية مقارنة بالتركيب الوراثي CC لصفات الإدرار في قطيع النطاف الجديد (6.3 مقابل 5.8 كجم لمحصول اللبن عند يوم الإختبار، 480 مقابل 380 جرام لمحصول الدهن عند يوم الإختبار ، 290 مقابل 230 جرام لمحصول البروتين عند يوم الإختبار، 2.43 مقابل 2.45 $\log^{10}$ لعدد الخلايا الجسمية باللبن عند يوم الإختبار) وكذلك كانت متوسطات المربعات الصغرى المعممة في قطيع النطاف القديم (6.3 مقابل 5.6 كجم لمحصول اللبن عند يوم الإختبار، 390 مقابل 350 جرام لمحصول الدهن عند يوم الإختبار، 290 مقابل 230 جرام لمحصول البروتين عند يوم الإختبار، 2.41 مقابل 2.45 $\log^{10}$ لعدد الخلايا الجسمية باللبن عند يوم الإختبار للتركيب الوراثي TC مقابل التركيب الوراثي CC في قطيع النطاف الجديد والنطاف القديم).

<u>الإرتباطات الجزيئية بين التراكيب الوراثية للجينات *GH* ،*FSHR* ، *PRL* وصفات التناسل</u>

أظهرت متوسطات المربعات الصغرى المعممة لصفات التناسل للتركيبين الوراثيين AA ، GG للجين *PRL* إرتباطات جزيئية معنوية بين التركيبين الوراثيين وصفات العمر عند اول ولادة، عدد الايام المفتوحة، الفترة بين ولادتين. وكانت الفروق في المتوسطات المعممة بين التركيبين الوراثيين AA ،GG معنوية للجين *PRL* لصفات العمر عند اول ولادة، عدد الأيام المفتوحة، الفترة

بين ولادتين معنوية وغالباً في صالح التركيب الوراثي GG مقارنة بالتركيبب الواثي AA في قطعان النطاف الجديد والنطاف القديم والجميزة. وكانت متوسطات المربعات الصغرى المعممة للتركيب الواثي GG تتراوح بين 33.8 إلى 41 شهرا لصفة العمر عند أول ولادة وبين 142 إلى 170 إلى يوماً لصفة عدد الايام المفتوحة و بين 435 إلى 469 يوماً لصفة الفترة بين ولادتين. وكانت المتوسطات المعممة في قطيع النطاف الجديد معنوية وفي صالح التركيب الوراثي GG للجين *PRL* لصفات التناسل مقابل التركب الواثي AA (41.0 مقابل 43.0 شهر لصفة العمر عند أول ولادة ، 142 مقابل 174 يوماً لصفة عدد الأيام المفتوحة، 345 مقابل 476 يوماً لصفة الفترة بين والادتين). وكذلك كانت المتوسطات المعممة في صالح التركيب الوراثي GG في قطيع النطاف القديم (33.8 مقابل 35.1 شهر لصفة العمر عند أول ولادة ، 143 مقابل 158 يوماً لصفة عدد الأيام المفتوحة ، 449 مقابل 459 يوماً لصفة الفترة بين ولادتين).

كانت الإختلافات بين المتوسطات المعممة لصفات التناسل للتراكيب الوراثية GG ،GC ، CC للجين *FSHR* في مختلف القطعان معنوية وفي صالح التركيب الوراثي CC . وكانت المتوسطات المعممة للتركيب الواثي CC للجين *FSHR* معنويا ومنخفضا عن المتوسطات المعممة للتركبين الوراثيين GC ، GG (37.9 مقابل 39.7 ،42.5 شهراً لصفة العمر عند أول ولادة ، 83 مقابل 91 ، 102 يوماً لصفة عدد الايام المفتوحة ، 387 مقابل 397 ، 419 يوماً لصفة الفترة بين ولادتين) في قطيع النطاف الجديد وكذلك في قطيع النطاف القديم (32.0 مقابل 34.3 ،35.6 شهرا لصفة العمر عند أول ولادة ، 83 مقابل 91 ، 102 يوماً لصفة عدد الأيام المفتوحة ، 384 مقابل 408 ، 398 يوماً لصفة الفترة بين ولادتين) وكذلك تم الحصول على إتجاهات مرغوبة في قطيع الجميزة (35.0 مقابل 37.3 ،36.5 شهراً لصفة العمر عند اول والادة ، 103 يوماً مقابل 109 ، 118 يوماً لصفة عدد الايام المفتوحة ، 366 يوماً مقابل 396 ، 410 يوماً لصفة الفترة بين ولادتين).

تم الكشف عن تركيبين وراثيين هما TC، CC للجين *GH* لصفات التناسل في قطعان النطاف الجديد والنطاف القديم والجميزة ،حيث كانت المتوسطات المعممة معنوية وفي صالح التركيب الوراثي TC . وكانت المتوسطات المعممة داخل كل قطيع من النطاف الجديد والنطاف القديم والجميزة هي 37.8 ، 33.7 ، 35.4 شهراً لصفة العمر عند اول والادة ، 93 ، 94 ، 105 يوماً لصفة عدد الايام المفتوحة ، 383 ، 379 ، 395 يوماً لصفة الفترة بين ولادتين مقابل 41.4 ، 35.2 ، 37.5 شهرا لصفة العمر عند اول والادة ، 115 ، 100 ، 121 يوماً لصفة عدد الايام المفتوحة ، 407 ، 393، 406 يوماً لصفة الفترة بين ولادتين.

الإرتباطات الجزيئية بين التراكيب الوراثية للجينات *GH* ، *FSHR* وصفات السائل المنوي

كانت الفروق في متوسطات المربعات الصغرى المعممة لصفات السائل المنوي بين التراكيب الوراثية GG ،GC ،CC للجين *FSHR* معنوية وفي صالح التركيب الوراثي GG حيث كانت الفروق في المتوسطات المعممة لصفات السائل المنوي بين التراكيب الوراثية الثلاثة للجين *FSHR* معنوية (2.9 ملي مقابل 2.6، 2.5 ملي لحجم القذفة المنوية، 64.1% مقابل 59.3، 63.2% لحركة الحيوانات المنوية، 62.8% مقابل 57.8، 61.9% لنسبة الحيوانات المنوية الحية، 9.1% مقابل 9.9، 9.4% لنسبة الحيوانات المنوية الشاذة، 1.59x 10^9 مقابل 1.36، 1.50 x10^9 لتركيز الحيوانات المنوية).

كانت الإرتباطات الجزيئية معنوية وفي صالح التركيب الوراثي CC مقابل التركيب الوراثي TC حيث كانت المتوسطات المعممة لصفات السائل المنوي في صالح التركيب الوراثي CC مقارنة بالتركيب الوراثي TC (2.9 ملي مقابل 2.5 ملي لحجم القذفة المنوية، 64.1% مقابل 60.9% لحركة الحيوانات المنوية، 62.1% مقابل 60.0% لنسبة الحيوانات المنوية الحية، 8.9% مقابل 9.6% لنسبة الحيوانات المنوية الشاذة، 1.60x 10^9 مقابل 1.40 x10^9 لتركيز الحيوانات المنوية).

الإرتباطات الجزيئية بين التراكيب الوراثية للجينات *FSHR* ،*PRL* ، *GH* وصفات النمو

كانت الإرتباطات الجزيئية معنوية وفي صالح التركيب الوراثي TC في قطعان النطاف الجديد، النطاف القديم والجميزة. كانت المتوسطات المعممة لصفات وزن الجسم ومعدل الزيادة اليومية في الوزن في جميع القطعان للتركيب الواثي TC أكبر معنوياً من المتوسطات للتركيب الواثي CC (36.8 مقابل 33.9 كجم للوزن عند الميلاد، 96.3 مقابل 91.8 كجم للوزن عند الفطام، 600 مقابل 540 جرام لمعدل الزيادة اليومية في الوزن). وفي كل قطيع تجريبي علي حده كانت المتوسطات المعممة لصفات الوزن عند الميلاد، الوزن عند الفطام، ومعدل الزيادة اليومية في الوزن أكبر وفي صالح التركيب الوراثي TC مقابل التركيب الوراثي CC (36.9 ، 94.8 كجم، 590 جرام مقابل 34.4 كجم، 91.2 كجم، 560 جرام في قطيع النطاف الجديد وكذلك 38.0 كجم، 95.9 كجم، 580 جرام مقابل 36.5 كجم، 90.9 كجم، 530 جرام في قطيع النطاف القديم، 39.0 كجم، 104.6 كجم، 660 جرام مقابل 33.0 كجم، 91.5 كجم، 530 جرام في قطيع الجميزة).

تم الكشف عن تركيبين وراثيين هما AA ،GG للجين البرولاكتين *PRL* (ثنائي النمط) في كل قطيع على حده(النطاف الجديد، النطاف القديم ،الجميزة). وكانت المتوسطات المعممة أكبر معنوياً لصفات الوزن ومعدل الزيادة اليومية وفي صالح التركيب الوراثي AA مقارنة بالتركيب الوراثي GG (38.6 مقابل 36.1 كجم للوزن عند الميلاد، 93.7 مقابل 90.8 كجم للوزن عند الفطام ،

594 مقابل 568 جرام لمعدل الزيادة اليومية في الوزن). على نفس السياق، كانت المتوسطات المعممة أكبر معنوياً لصفة الوزن عند الميلاد، لصفة الوزن عند الفطام ، لصفة معدل الزيادة اليومية في الوزن لصالح التركيب الوراثي AA مقابل التركيب الوراثي GG (43.9 كجم، 95.9 كجم، 594 جرام مقابل 33.9 كجم، 92.6 كجم، 472 جرام في قطيع النطاف الجديد، 36.4 كجم، 95.0 كجم، 605 جرام مقابل 34.0 كجم، 90.8 كجم، 542 جرام في قطيع النطاف القديم، 43.7 كجم، 99.9 كجم، 623 جرام مقابل 34.8 كجم، 77.6 كجم، 367 جرام في قطيع الجميزة).

تم الكشف عن ثلاث تراكيب وراثية هي GG، GC، CC للجين *FSHR* (متعدد الأنماط) حيث كانت الفروق في المتوسطات المعممة معنوية لصفات الوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن وفي صالح التركيب الوراثي GG مقارنة بالتراكيب الوراثية CC، GC. كانت المتوسطات المعممة في جميع القطعان أكبر معنوياً لصفات الوزن ومعدل الزيادة اليومية في الوزن لصالح التركيب الوراثي GG مقارنة بالتراكيب الوراثية GC، CC (38.6 مقابل 35.4، 36.8 كجم للوزن عند الميلاد، 97.9 مقابل 92.8، 92.6 كجم للوزن عند الفطام، 603 مقابل 558، 452 جرام لمعدل الزيادة اليومية في الوزن). في كل من قطعان النطاف الجديد والنطاف القديم وجد أن متوسطات المربعات الصغرى المعممة أكبر معنوياً لصفات الوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن لصالح التركيب الوراثي GG مقارنة بالتراكيب الوراثية CC، GC (37.7 كجم، 102.9 كجم، 662 جرام للتركيب الوراثي GG مقابل 32.9 كجم، 89.1 كجم، 550 جرام للتركيب الوراثي GC وكذا 36.3 كجم، 88.8 كجم، 524 جرام للتركيب الوراثيCC في قطيع النطاف الجديد، 38.1 كجم، 95.3 كجم، 577 جرام للتركيب الوراثيGG مقابل 36.5 كجم، 92.8 كجم، 552 جرام للتركيب الوراثيGC وكذا 36.5 كجم، 92.5 كجم، 537 جرام للتركيب الوراثي CC في قطيع النطاف القديم). وعلي النقيض كانت المتوسطات المعممة أكبر معنوياً للوزن عند الميلاد والوزن عند الفطام ومعدل الزيادة اليومية في الوزن لصالح التركيب الوراثي CC في قطيع الجميزة مقارنة بالتراكيب الوراثية GG، GC (39.4 كجم، 106.2 كجم، 636 جرام للتركيب الوراثي CC مقابل 33.3 كجم، 93.3 كجم، 574 جرام للتركيب الوراثي GC وكذا 35.5 كجم، 92.5 كجم، 548 جرام للتركيب الوراثي GG).

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