

USING GWAS (GENOME WIDE ASSOCIATION STUDIES) FOR GENETIC IMPROVEMENT OF ECONOMIC CHARACTERS IN BUFFALO: A REVIEW ARTICLE

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SUMMARY

Traditional progeny testing programs practiced on cattle in the developed countries require a lot of time and expense to be practiced for buffalo in the developing countries. Presently, GWAS (genome wide association studies) for economic characters in buffalo are still very limited compared to dairy cattle. This genomic method makes use of the entire genome to identify the genes associated with economically significant traits. The recent availability of medium density SNP platform (90K array) for buffalo facilitated the integration of genomic information and the phenotypic data to improve the accuracy of the individual buffalo evaluation and to accelerate the genetic improvement program. The present article provides the methodology and applications of GWAS in buffalo in terms of: 1) Website searching for candidate genes associated with lactation, reproduction, semen, growth and carcass traits, 2) Goals and uses of QTL and GWAS approaches, 3) The SNP chips and genetic markers used for GWAS analyses, 4) Genomic databases and Software used in GWAS analyses, 5) The methodology to be performed in GWAS analyses, 6) Implementing single-step GWAS to analyze GWAS dataset, 7) Reliability of GWAS analyses, 8) The advantages of ssGBLUP over classical GWAS, 9) Plotting the Manhattan Figures from GWAS results, 10) Putative candidate genes identified by GWAS analyses in the Egyptian buffalo studies, 11) Candidate and novel genes identified by GWAS analyses in non-Egyptian buffalo studies, 12) Estimating Genomic Breeding Values (GEBV) and Genomic selection (GS), and 13) Highlighted conclusions concerning GWAS.

Keywords: Egyptian buffalo, Genome Wide Association Studies, Candidate genes, Estimated Genomic Breeding Values, Genomic selection.

INTRODUCTION

The implementation of molecular methodologies within genetic improvement initiatives for livestock has the potential to decrease the expenses associated with breeding programs by approximately 92% (Schaeffer, 2006) and to augment the rate of genetic advancement twofold (De Roos *et al.*, 2011). The most common studies concerning the candidate genes associated with economic traits in buffalo were performed in China, India, Pakistan, Italy, Egypt, Brazil, Iran, and Turkiye. In this concept, several GWAS were conducted to identify buffalo candidate genes for lactation traits (de Camargo *et al.*, 2015; El-Halawany *et al.*, 2017; Liu *et al.*, 2018; Deng *et al.*, 2019; Abdel-Shafy *et al.*, 2020a&b; Liu *et al.*, 2020; Rehman *et al.*, 2021; Chhotaray *et al.*, 2023, and Lázaro *et al.*, 2023). Oppositely, few GWAS on buffalo concerning candidate genes were performed for reproductive and fertility traits (de Camargo *et al.*, 2015; Li *et al.*, 2018; Vohra *et al.*, 2021; Shao *et al.*, 2021; Mohammadi *et al.*, 2022, and Rehman *et al.*, 2022), for semen traits (EL Nagar *et al.*, 2023) and for growth traits (Guzman *et al.*, 2020; Rehman

et al., 2021, and Khan *et al.*, 2022). The Quantitative Trait Loci (QTLs) associated with milk production and semen traits in Egyptian buffalo were previously identified by microsatellite markers or GWAS methodology (El-Halawany *et al.*, 2017; Rushdi *et al.*, 2017; Abdel-Shafy *et al.*, 2020a,b; Awad *et al.*, 2020, and EL Nagar *et al.*, 2023) and in Non-Egyptian buffalo (de Camargo *et al.*, 2015; Da Costa Barros *et al.*, 2018; Liu *et al.*, 2018; Herrera *et al.*, 2018; Deng *et al.*, 2019; Lu *et al.*, 2020; Vohra *et al.*, 2021, and Lázaro *et al.*, 2023).

Egyptian and Italian buffalo are classified as Mediterranean river buffalo (*Bubalus bubalis*), possess a total of 50 chromosomes (25 pairs) (Minervino *et al.*, 2020). These river buffalo also have achieved elevated standards of productivity in China and various Southeast Asian nations, because of selective breeding practices, while the majority of other buffalo breeds are classified as swamp types, characterized by having 24 pairs of chromosomes, and they exhibit suboptimal milk production capabilities (Mishra *et al.*, 2015). In Egypt, however, the absence of national recording systems and adequate pedigree data make it is

difficult to implement conventional breeding strategies for improving the Egyptian buffalo (Abdel-Shafy *et al.*, 2020a,b). Such national recording system would be difficult to implement under the current Egyptian animal farming conditions due to that over 85% of the Egyptian buffalo population are owned by small holders who having small herd sizes.

Liu *et al.* (2018) recognized five genes (*MFSD14A*, *SLC35A3*, *PALMD*, *RGS22*, and *VPS13B*) as innovative candidate genes for milk production. In recent years, GWAS have emerged as a crucial approach for investigating complex traits in buffalo and these studies have played pivotal roles in sustainable development of buffalo production through exploring the genetic diversity, detecting the candidate genes and promoting the animal genetic improvement and breeding methods (Deng *et al.*, 2019; Abdel-Shafy *et al.*, 2020a,b; Liu *et al.*, 2020; Pacheco *et al.*, 2020; Rehman *et al.*, 2021; Shao *et al.*, 2021; EL Nagar *et al.*, 2023, and Lázaro *et al.*, 2023). Thus, understanding the

genomic architectures for lactation, reproduction, semen and growth traits is essential for genetic improvement in buffalo programs (Moaen-ud Din and Bilal, 2016, and EL Nagar *et al.*, 2023).

Website searching for candidate genes associated with lactation, reproduction, semen and growth traits in buffalo:

The knowledge in the present article is dealing with the identification of candidate genes associated with lactation, reproduction, behavior, heat stress, semen and growth traits (Mishra *et al.*, 2013, and Khalil, 2020). The National Center for Biotechnology Information (NCBI) database was used to find out the genes closely associated with economic traits in buffalo. The candidate genes associated significantly with lactation characters are summarized for Egyptian (Table 1) and non-Egyptian (Table 2) buffalo. Several genes molecularly associated with milk yields and components were detected on chromosomes numbers 1, 2, 3, 4, 7, 8, 15, 19 and 20.

Table 1. The candidate genes molecularly associated with milk production and composition traits detected in Egyptian buffalo

CN*	Candidate gene	N	Reference
1	Pituitary-specific transcription factor-1 (<i>Pit1</i>)	100	Othman <i>et al.</i> (2012)
2	Prolactin (<i>PRL</i>)	100	Othman <i>et al.</i> (2012)
		200	El-Magd <i>et al.</i> (2015)
		400	El-Komyet <i>et al.</i> (2020)
		266	Abd El Fattahet <i>et al.</i> (2023)
3	Growth hormone (<i>GH</i>)	100	Othman <i>et al.</i> (2012)
		400	El-Komyet <i>et al.</i> (2020)
4	Insulin like growth factor 1 (<i>IGF-1</i>)	400	El-Komyet <i>et al.</i> (2020)
		99	Ali <i>et al.</i> (2022)
7	Kappa-Casein gene (<i>CSN</i>)	100	Othman <i>et al.</i> (2012)
		340	Al-Shawa <i>et al.</i> (2019)
		400	El-Komyet <i>et al.</i> (2020)
8	Leptin gene (<i>LEP</i>)	200	Abdo <i>et al.</i> (2014)
		81	Mahrous <i>et al.</i> (2020)
		99	Ali <i>et al.</i> (2022)
15	Diacylglycerol O-acyltransferase 1 gene (<i>DGATI</i>)	400	El-Komy <i>et al.</i> (2020)
19	Growth hormone receptor (<i>GHR</i>)	100	Othman <i>et al.</i> (2012)
	Prolactin-like receptor gene (<i>PRLR</i>)	400	El-Komy <i>et al.</i> (2020)
		400	El-Magd <i>et al.</i> (2021)
20	Insulin like growth factor 1 receptor (<i>IGF-1R</i>)	266	Abd El Fattahet <i>et al.</i> (2023)
		99	Ali <i>et al.</i> (2022)

*CN= Chromosome number

Table 2. The candidate genes molecularly associated with lactation characters detected in non-Egyptian buffalo

CN*	Candidate gene	Buffalo breed	N	Reference
1	Melatonin receptor 1A (<i>MTRN1A</i>) Pituitary-specific transcription factor-1 (<i>Pit1</i>)	Brazilian	140	Machado <i>et al.</i> (2016), Brazil
		Amazon	180	Barbosa <i>et al.</i> (2017), Brazil
		Murrah	100	Mavi <i>et al.</i> (2017), India
		Khuzestan	60	Ahmadzadeh <i>et al.</i> (2019), Iran
		Anatolian	35	Toparslan <i>et al.</i> (2019), Turkey
2	Prolactin (<i>PRL</i>)	Indian	96	Ladani <i>et al.</i> (2003), India
		Indian	50	Madnalwaret <i>et al.</i> (2010), India
		Italian	462	Li <i>et al.</i> (2017), China
		Mediterranean	50	Madnalwaret <i>et al.</i> (2010), Italy
		Murrah	100	Mavi <i>et al.</i> (2017), India
		Anatolian	126	Konca and Akyüz. (2017), Turkey
		Anatolian	129	Özşensoy (2018), Turkey
		Anatolian	35	Toparslan <i>et al.</i> , (2019), Turkey
		Anatolian	220	Araújo <i>et al.</i> (2015), Brazil
3	Adrenoceptor alpha 1A (<i>ADRA1A</i>) Fatty acid synthase (<i>FASN</i>) Growth hormone (<i>GH</i>)	Brazilian	220	Araújo <i>et al.</i> (2015), Brazil
		Murrah	162	Kumar <i>et al.</i> (2017), India
		Khuzestan	60	Ahmadzadeh <i>et al.</i> (2019), Iran
4	Alpha-2-macroglobulin (<i>A2M</i>)	Murrah	36	Freitas <i>et al.</i> (2016), Brazil
		Murrah	36	Ramesha <i>et al.</i> (2015), India
		Surti	36	Ramesha <i>et al.</i> (2015), India
6	Casein alpha S2 (<i>CSNS2</i>)	Bhadawari	NA	Misra <i>et al.</i> (2008), India
		Murrah	NA	Misra <i>et al.</i> (2008), India
		Mehsana	NA	Misra <i>et al.</i> (2008), India
		Surti	NA	Misra <i>et al.</i> (2008), India
		Surti	NA	Misra <i>et al.</i> (2008), India
7	Kappa-casein N3 (<i>CSN3</i>) Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (<i>PPARGC1A</i>)	Murrah	115	Otaviano <i>et al.</i> (2005) , Brazil
		Khuzestan	60	Ahmadzadeh <i>et al.</i> (2019), Iran
		Anatolian	35	Toparslan <i>et al.</i> , (2019), Turkey
		Italian	NA	Hosseini <i>et al.</i> (2021), China
		Mediterranean	NA	Hosseini <i>et al.</i> (2021), China
		Murrah	60	Sihag <i>et al.</i> (2023), India
		Bhadawari	40	Sihag <i>et al.</i> (2023), India
		Murrah	36	Ramesha <i>et al.</i> (2015), India
		Surti	36	Ramesha <i>et al.</i> (2015), India
		Mehsana	154	Tanpure <i>et al.</i> (2012), India
8	Insulin-like growth factor binding protein-3 (<i>IGFBP-3</i>) Leptin gene (<i>LEP</i>)	Marathwada	30	Tanpure <i>et al.</i> (2012), India
		Chilika	24	Tanpure <i>et al.</i> (2012), India
		Jaffarabadi	19	Tanpure <i>et al.</i> (2012), India
		Murrah	15	Tanpure <i>et al.</i> (2012), India
		Nili-Ravi	15	Tanpure <i>et al.</i> (2012), India
		Toda	15	Tanpure <i>et al.</i> (2012), India
		Pandharpuri	15	Tanpure <i>et al.</i> (2012), India
		Nagpuri	14	Tanpure <i>et al.</i> (2012), India
		Brazilian	174	Zetouni <i>et al.</i> (2013), Brazil
		Murrah	154	Jamuna <i>et al.</i> (2016), India
		Anatolian	54	Kaplan (2018), Turkey
		Murrah,	69	Silva <i>et al.</i> (2021), Brazil
		Mediterranean	69	Silva <i>et al.</i> (2021), Brazil
		Mediterranean	322	Pauciuilloet <i>et al.</i> (2012a), Italy
		Italian	322	Pauciuilloet <i>et al.</i> (2012a), Italy
		Brazilian	220	Araújo <i>et al.</i> (2015), Brazil
14	Oxytocin/neurophysin (<i>OXT</i>) Growth hormone releasing hormone (<i>GHRH</i>)	Khuzestan	60	Ahmadzadeh <i>et al.</i> (2019), Iran
		Khuzestan	60	Ahmadzadeh <i>et al.</i> (2019), Iran

Table 2. Cont.

CN*	Candidate gene	Buffalo breed	N	Reference
15	Diacylglycerol O-acyltransferase 1 (<i>DGATI</i>)	Chinese	117	Yuan <i>et al.</i> (2007), China
		Anatolian	41	Özdil and İlhan (2012), Turkey
		Mazandaran,	25	Heydarian <i>et al.</i> (2014), Iran
		Khuzestan,	52	Heydarian <i>et al.</i> (2014), Iran
		Guilan,	25	Heydarian <i>et al.</i> (2014), Iran
		Azerbaijan	119	Heydarian <i>et al.</i> (2014), Iran
		Kermanshah	19	Heydarian <i>et al.</i> (2014), Iran
		Mehsana	130	Parikh <i>et al.</i> (2016), India
		Italian	462	Li <i>et al.</i> (2018), China
		Murrah	110	Sulabh <i>et al.</i> (2018), India
		Iraqi	60	Kadhim and Ibrahim (2019), Iraq
		Murrah	157	Krovvidi <i>et al.</i> (2021), India
		Mediterranean	31	Khan <i>et al.</i> (2021), India
19	Casein alpha S1 (<i>CSNSI</i>)	Bhadawari	NA	Misra <i>et al.</i> (2008), India
		Murrah	NA	Misra <i>et al.</i> (2008), India
		Mehsana	NA	Misra <i>et al.</i> (2008), India
		Surti	NA	Misra <i>et al.</i> (2008), India
	Growth hormone receptor (<i>GHR</i>)	Murrah	36	Ramesha <i>et al.</i> (2015), India
		Surti	36	Ramesha <i>et al.</i> (2015), India
		Anatolian	329	Erdoğan <i>et al.</i> (2021), Turkey
	Prolactin-like receptor (<i>PRLR</i>)	Murrah	32	Al-Kal <i>et al.</i> (2018), China
		Nili-Ravi	31	Al-Kal <i>et al.</i> (2018), China
		Jaffarabadi	50	Devkatte <i>et al.</i> (2021), India
21	Oxytocin receptor (<i>OXTR</i>)	Surti	50	Devkatte <i>et al.</i> (2021), India
		Italian	306	Cosenza <i>et al.</i> (2017), Italy
	Ghrelin (<i>GHRL</i>)	Mediterranean		
		Brazilian	240	Gil <i>et al.</i> (2013), Brazil
	Lactoferrin (<i>LTF</i>)	Italian	322	Pauciullo <i>et al.</i> (2015), Italy
22	Melanocortin 4 receptor (<i>MC4R</i>)	Mediterranean		
		Chinese	332	Deng <i>et al.</i> (2016), China
	Stearoyl-CoA desaturase (<i>SCD</i>)	Murrah	174	Singh <i>et al.</i> (2019), India
		Italian	322	Pauciullo <i>et al.</i> (2012b), Italy
		Mediterranean		
		Murrah,	69	Silva <i>et al.</i> (2021), Brazil
		Mediterranean		

*CN= Chromosome number, NA= Not Available

The candidate genes associated significantly with reproduction traits of Egyptian and non-Egyptian buffalo are presented in Table 3. Several genes molecularly associated with reproductive traits such as stillbirth, calving ease, gestation length, postpartum interval to pregnancy, calving interval, and age at first calving (de Araujo Neto, 2020, and Abd ElFattah *et al.*, 2023) were detected on chromosomes number 1, 2, 4, 12, 14, 19 and 22.

Low heritability estimates for semen traits in buffalo made it difficult to perform traditional direct selection for most of the reproductive traits. Several studies on cattle semen (Mishra *et al.*, 2013; Suchocki and Szyda, 2015; Qin *et al.*, 2016; Yin *et al.*, 2019; Pacheco *et al.*, 2020; Butler *et al.*, 2022; Modiba *et al.*, 2022; Abril-Parreño, *et al.*, 2023, and Ramirez-Diaz *et al.*, 2023) but rare studies

on buffalo (Khan *et al.*, 2022, and EL Nagar *et al.*, 2023) have been identified for the genes and genetic markers associated with semen specifications. Therefore, it seems to be very important to identify the whole bunch of genes that are associated with semen quality traits to enable selecting the buffalo bulls excellent for sperms quality traits. In GWAS analyses, several candidate genes such as *GDF9*, *FSHB*, *GNRHR*, *LHCGR*, *INHA*, *PRL*, *BMP15*, *TNP2*, *CFTR*, *IGF1R*, *CATSPER1*, *CAPN1* and *KATNA1* have been identified to impact sperm characteristics and function ability (Qin *et al.*, 2016). During the last decade, few studies have shown significant associations of certain candidate genes with semen traits in Chinese Holstein bulls (Qin *et al.*, 2016) and in buffalo (Table 4).

Table 3. The candidate genes molecularly associated with reproductive traits as cited in buffalo literature

CN*	Candidate gene	N	Buffalo breed	Reference
1	Melatonin receptor 1A (<i>MTRN1A</i>)	167	Brazilian	Zetouni <i>et al.</i> (2014), Brazil
2	Prolactin (<i>PRL</i>)	266	Egyptian	Abd El Fattah <i>et al.</i> (2023), Egypt
		50	Indian	Madnalwar <i>et al.</i> (2010), India
12	Follicle-stimulating hormone receptor (<i>FSHR</i>)	100	Egyptian	Othman and Abd-el Samad (2013), Egypt
		150	Egyptian	Sosa <i>et al.</i> (2015), Egypt
		60	Egyptian	ShafiKet <i>et al.</i> (2017), Egypt
		203	Murrah	Kathiravan <i>et al.</i> (2019), India
		50	Egyptian	Ramadan <i>et al.</i> (2020), Egypt
		243	Egyptian	Fouda <i>et al.</i> (2021), Egypt
		50	Egyptian	Sallam <i>et al.</i> (2022), Egypt
		90	Egyptian	Wagdy <i>et al.</i> (2023), Egypt
	Luteinizing hormone receptor (<i>LHR</i>)	100	Egyptian	Othman and Abd-el Samad (2013), Egypt
		301	Egyptian	Sosa <i>et al.</i> (2016), Egypt
	Estrogen receptor- α (<i>ERα</i>)	100	Egyptian	Othman and Abd-el Samad (2013), Egypt
19	Prolactin-like receptor (<i>PRLR</i>)	266	Egyptian	Abd El Fattah <i>et al.</i> (2023), Egypt
22	Lactoferrin (<i>LTF</i>)	400	Egyptian	El-Magd <i>et al.</i> (2021), Egypt
		150	Egyptian	El-Debaky <i>et al.</i> (2020), Egypt

*CN= Chromosome number

Table 4. The candidate genes molecularly associated with semen traits as cited in buffalo literature

CN*	Candidate gene	N	Buffalo breed	Reference
1	Pituitary-specific transcription factor 1 (<i>PIT-1</i>)	60	Egyptian	Hasanain <i>et al.</i> (2016), Egypt;
		35	Anatolian	Toparslan <i>et al.</i> (2019), Turkey
	Sperm associated antigen 11B (<i>SPAG11B</i>)	130	Murrah	Deshmukh <i>et al.</i> (2021), India
2	Transition nuclear protein-1 (<i>TNP-1</i>)	60	Murrah	Panigrahi and Yadav (2010), India
	Capping actin protein Z-line beta subunit (<i>CAPZB</i>)	10	Murrah	Xiong <i>et al.</i> (2018), China
	Heat shock protein70 (<i>HSP70</i>)	37	Egyptian	Gafer <i>et al.</i> (2015), Egypt
	Prolactin (<i>PRL</i>)	60	Egyptian	Hasanain <i>et al.</i> (2017), Egypt
3	Growthhormone (<i>GH</i>)	NA	Egyptian	Darwish <i>et al.</i> (2016), Egypt
	Prohibitin (<i>PHB</i>)	10	Murrah	Xiong <i>et al.</i> (2018), China
	Aquaporin 7 (<i>AQP7</i>)	21	Murrah	Kumari <i>et al.</i> (2018a), India
		69	Murrah	Kumari <i>et al.</i> (2018b), India
		69	Surti	Kumari <i>et al.</i> (2018c), India
	Gonadotropin-releasing hormone (<i>GnRH</i>)	NA	Chinese	Wang <i>et al.</i> (2020), China
6	Tektin-2 (<i>TEKT2</i>)	10	Murrah	Xiong <i>et al.</i> (2018), China
7	Gonadotropin releasing hormone receptor (<i>GnRHR</i>)	60	Chinese	Wang <i>et al.</i> (2017), China
		50	Egyptian	Mahmoud <i>et al.</i> (2021), Egypt
11	AT-rich interaction domain 4A (<i>ARID4A</i>)	156	Chinese	Lu <i>et al.</i> (2022), China
12	Luteinizing hormone receptor (<i>LHR</i>)	NA	Murrah	Reen <i>et al.</i> (2018), India
16	Ubiquilin-3	115	Egyptian	EL Nagar <i>et al.</i> (2023), Egypt
17	Inhibit alpha (<i>INHA</i>)	103	Murrah	Chandra <i>et al.</i> (2020), India.
18	Luteinizing hormone beta (<i>LHβ</i>)	NA	Murrah	Reen <i>et al.</i> (2018), India
X	Melanoma-associated antigen 2, Cancer/testis antigen 47A-like, Actin-related protein T1, Sodium/hydrogen exchanger 2-like.	115	Egyptian	EL Nagar <i>et al.</i> (2023), Egypt

*CN= Chromosome number, NA= Not Available

Strong associations of some candidate genes with body weights and gains and carcass and meat

composition traits were reported in several buffalo studies (Table 5). Such candidate genes along with

those listed in GWAS analyses (Guzman *et al.*, 2020; Pagala *et al.*, 2020, and Khan *et al.*, 2022)

were significantly associated with growth and carcass traits in buffalo.

Table 5. The candidate genes molecularly associated with growth, carcass and meat composition traits listed in buffalo literature

CN*	Candidate gene	N	Buffalo breed	Reference
1	Pituitary-specific transcription factor-1 (<i>Pit1</i>)	100	Egyptian	Othman <i>et al.</i> (2012), Egypt
		60	Khuzestan	Ahmadzadeh <i>et al.</i> (2019), Iran
2	Prolactin (<i>PRL</i>)	266	Egyptian	Abd El Fattah <i>et al.</i> (2023), Egypt
	Myostatin (<i>MSTN</i>)	NA	Murrah	Páez <i>et al.</i> (2021), Colombia
3	Growth hormone (<i>GH</i>)	452	Indonesian	Andreas <i>et al.</i> (2010), Indonesia
		126	Anatolian	Konca and Akyüz. (2017), Turkey
		60	Khuzestan	Ahmadzadeh <i>et al.</i> (2019), Iran
		45	Simeulue	Eriani <i>et al.</i> (2019), Indonesia
		150	Anatolian	Özkan <i>et al.</i> (2020), Turkey
		58	Swamp	Nafiu <i>et al.</i> (2020), Indonesia
7	Kappa-Casein N3 (<i>KCN3</i>)	60	Khuzestan	Ahmadzadeh <i>et al.</i> (2019), Iran
8	Leptin (<i>LEP</i>)	100	Egyptian	Othman <i>et al.</i> (2011), Egypt
		54	Anatolian	Kaplan (2018), Turkey
10	Insulin-like growth factor 2 receptor (<i>IGF2R</i>)	200	Egyptian	El-Magd <i>et al.</i> (2014), Egypt
	Insulin-like growth factor 1 receptor (<i>IGF1R</i>)	200	Egyptian	El-Magd <i>et al.</i> (2013), Egypt
14	Growth hormone releasing hormone (<i>GHRH</i>)	200	Egyptian	El-Magd <i>et al.</i> (2017), Egypt
		36	Murrah	Ramesha <i>et al.</i> (2015), India
		126	Anatolian	Konca and Akyüz. (2017), Turkey
		60	Khuzestan	Ahmadzadeh <i>et al.</i> (2019), Iran
		58	Swamp	Nafiu <i>et al.</i> (2020), Indonesia
17	Inhibin-βA (<i>INHβA</i>)	NA	Philippine	Babera <i>et al.</i> (2022), Philippine
19	Growth hormone receptor (<i>GHR</i>)	452	Indonesian	Andreas <i>et al.</i> (2010), Indonesia
		329	Anatolian	Erdoğan <i>et al.</i> (2021), Turkey
	Prolactin-like receptor (<i>PRLR</i>)	400	Egyptian	El-Magd <i>et al.</i> (2021), Egypt
		266	Egyptian	Abd El Fattah <i>et al.</i> (2023), Egypt

*CN= Chromosome number, NA= Not Available

Goals and uses of QTL and GWAS approaches in buffalo:

In the last few decades, the chromosomal regions of genes affecting production and fitness traits in buffalo were mapped using linkage analysis and linkage disequilibrium (LD) between markers and QTLs. The accuracies of buffalo genomic investigations are compromised if the existing genetic reference map is not used properly (Amaral *et al.*, 2008; Venturini *et al.*, 2014; Abdel-Shafy *et al.*, 2020a, and EL Nagar *et al.*, 2023). Wu *et al.* (2013) revealed that out of 935 SNPs linked to buffalo milk performance only, seven were spotted using the SNP50 Bead Chip (Illumina Inc., San Diego, CA). At that time, QTL mapping was largely based on microsatellite markers (Liu *et al.*, 2018). Rushdi *et al.* (2017) illustrated that QTL using microsatellite marker scan be utilized to identify the candidate genes underlying milk production traits in buffalo. Although microsatellite markers, as used by Rushdi *et al.* (2017), contributed valuable information, their limited density and genome coverage restrict their effectiveness in identifying candidate genes for complex traits. In contrast, current genomic approaches such as SNP arrays and next-generation sequencing technologies offer higher resolution and

more reliable frameworks for gene and QTL detection and identifying the markers, genomic regions and candidate genes associated with several economically important traits across the entire genome in buffalo, *e.g.* lactation, reproductive, semen, carcass, meat composition and growth traits (Abdel-Shafy *et al.*, 2020a; EL Nagar *et al.*, 2023; Lázaro *et al.*, 2023, and Ren *et al.*, 2024).

With the completion of buffalo genome sequencing project (Yates *et al.*, 2020) and the advancement in genotyping technologies in buffalo (Lázaro *et al.*, 2023), GWAS was rarely used in the detection of the genetic variants that affect milk production and various quantitative traits. GWAS in buffalo has gained popularity in mapping QTL for the economically important traits like calving ease, milk yield, fat and protein percentage, fertility traits, meat quality and quantity, sensory panel evaluation... etc. Accordingly, GWAS proved to be an ideal method to identify genes associated with various phenotypes and to elucidate the mechanisms of the complex traits in buffalo (Sharma *et al.*, 2015). In practice, GWAS possess some powerful statistical-genetic tools that aid buffalo genomic studies, and it was successfully employed to identify the promising candidate genes for key traits in buffalo (Lázaro *et al.*,

2023). Currently, GWAS approach has gained popularity in identifying the genes associated with various complex traits in buffalo (Sharma *et al.*, 2015, and Abdel-Shafy *et al.*, 2020a). It has significant advancements in genetic research and breeding practices (Tan *et al.*, 2023). GWAS is an effective alternative approach to find genomic regions associated with economic characters and to integrate all phenotypes from genotyped and non-genotyped animals and pedigree information in a Single-Step of Genomic Best Linear Unbiased Prediction framework (ssGBLUP) (Aguilar *et al.*, 2019, and Misztal *et al.*, 2018). The genomic estimated breeding values (GEBVs) are transformed into marker effects and marker weights using the ssGBLUP. The solutions of mixed model equations are then updated iteratively (Misztal *et al.*, 2018). The main goals and uses for GWAS as cited in buffalo literature are summarized as: 1) Determining the connection between the characters of interest and particular molecular markers (SNPs or genomic areas) (Seidel, 2009, and Lu *et al.*, 2011), 2) Pinpointing the genetic loci having molecular associations with economic traits, 3) Providing the breeding and stakeholders with selection tools to enhance the economic buffalo traits such as lactation, growth, reproduction, and meat quality (Suchocki and Szyda, 2015; Abdel-Shafy *et al.*, 2020a; EL Nagar *et al.*, 2023; Lázaro *et al.*, 2023, and Ren *et al.*, 2024), 4) Enabling rapid advancements in genotyping of bovine animals using the technique of SNP (Lu *et al.*, 2011; Liu *et al.*, 2018, and Vohra *et al.*, 2021), 5) Identifying the genetic makeup of complex features and the relationships between genotype and phenotype in a variety of buffalo breeds (Lázaro *et al.*, 2023), 6) Contributing in gene editing and functional biology (Lu *et al.*, 2020), 7) Improving the efficiency of genomic selection (Hayes *et al.*, 2009; Van der Werf, 2013; Liu *et al.*, 2018; Cesarani *et al.*, 2021a,b, and Tan *et al.*, 2023), 8) Highlighting the details of disease susceptibilities and the genetic architecture of complex traits (Zhang *et al.*, 2015), 9) Focusing comprehensively on surveys of variants across the genome to identify the genes associated with a certain phenotype in buffalo (Sharma *et al.*, 2015, and Lázaro *et al.*, 2023), 11) Integrating the phenotypes with genotypes through using pedigree and genomic information in framework of ssGBLUP (Misztal *et al.*, 2018; Yin *et al.*, 2019, and Ma *et al.*, 2023), 12) Facilitating precisely the identification of QTL by examining the associations between genetic markers and the phenotypic values of the individuals, leading to the succession of the breeding programs in buffalo.

SNP chips and genetic markers used for GWAS analyses in buffalo:

Since 2006, high-density SNP panels have been used in livestock genomics, and they have been shown to carry out and greatly improve genomic

selection in dairy cows (Hayes *et al.*, 2009; Seidel, 2009; Lu *et al.*, 2011; Penagaricano *et al.*, 2012; Segura *et al.*, 2012; Van der Werf, 2013, and Abril-Parreño *et al.*, 2023). Later, Illumina, Neogen (previously Geneseek), and Affymetrix produced commercial SNP arrays, each of which provided platforms of genotyping SNPs at different densities (Borquis *et al.*, 2014). Since cattle and buffalo share chromosomes, the bovine SNP chip has been used in GWAS to study the genomics of buffalo milk production traits (Wu *et al.*, 2013; Venturini *et al.*, 2014; de Camargo *et al.*, 2015; Cesarani *et al.*, 2021a,b, and Abril-Parreño *et al.*, 2023). For the first time, Wu *et al.* (2013) identified seven SNPs associated with milk yield in buffalo populations by using a bovine SNP chip (Illumina Bovine SNP 50 Bead Chip). Then, Venturini *et al.* (2014) applying a high-density bovine SNP chip on Brazilian buffalo (777 k SNPs, Illumina Infinium Bovine HD Bead Chip) reported that only two SNPs on chromosomes 15 and 20 were confirmed to have significant association with milk yield. In the last few years, Affymetrix (Axiom Buffalo Genotyping Array 90 K) was released as a commercial buffalo SNP chip and therefore, the SNP markers were used in GWAS analysis (Du *et al.*, 2019, and Abril-Parreño *et al.*, 2023). According to the buffalo SNP array, 78 SNPs have a significant impact on the production and content of milk in buffalo (Iamartino *et al.*, 2013; de Camargo *et al.*, 2015, and El-Halawany *et al.*, 2017). Using GWAS, it was discovered that several genetic areas on chromosomes 1, 5, and 6 are linked to daily milk production in Egyptian buffalo (El-Halawany *et al.*, 2017). According to Tan *et al.* (2023), genomic selection (GS) uses two procedures to find the genetic markers underpinning complex traits:

- 1) Single-step genomic best linear unbiased prediction (ss-GBLUP) is the basis for single-step GWAS. According to several models, including animal models with a single or multiple trait(s), this approach is the most popular for estimating the impact of a SNP on individuals with or without phenotypic values (Yin *et al.*, 2019, and Ma *et al.*, 2023).
- 2) The BLUP genetic architecture (BLUP|GA). Using this approach, the weighted sum genetic architecture matrix (GA), a genomic relatedness matrix, and covariance matrix data are combined. According to Zhang *et al.* (2015), the GA matrix is computed using the prior data for unique traits that GWAS has collected.

Software used in GWAS analyses:

Many free software tools are available for GWAS analysis, such as Gen A Map (<http://cogito-b.ml.cmu.edu/genamap>), R GenABEL (<https://cran.r-project.org/src/contrib/Archive/GenABEL>, Aulche nko *et al.*, 2007), and PLINK (<http://pngu.mgh.harvard.edu/purcell/plink>, Purcell *et al.*, 2007). These technologies are crucial for

structured association mapping, linkage disequilibrium (LD), and quality control stratification (Hayes *et al.*, 2003). Additionally, population stratification is done using GEMMA (Genome-wide Efficient Mixed Model Association) for association mapping, heritability estimate, and identical by descent (IBD) analysis <http://www.xzlab.org/software.html>. By using data conditioning, variance estimation techniques, and SNP data to improve accuracy of breeding value estimation, BLUPF90 makes GWAS methodology easier to handle (<http://nce.ads.uga.edu/wiki/doku.php?id=documentation>).

The methodology to be performed in GWAS analyses for buffalo:

GWAS analysis is executed according to the following steps:

- (1) As starting point, the Expected Breeding Values (EBV) are to be estimated for the animals using their phenotypic data set and the complete pedigree using univariate animal model of BLUPF90 software (Misztal *et al.*, 2018). The applied univariate animal model to be used is:

$$y = Xb + Z_a u_a + Z_p u_p + e$$

Where b represents the vector of all fixed effects, incorporating the effects of parity and test day order, the combined effect of herd, year and season of calving, linear regressions of age at calving and days in milk, vectors of a and p represent random additive genetic and permanent environmental effects, respectively, whereas e denotes the vector of random residuals. X , Z_a , and Z_p = incidence matrices relate to fixed effects, random animal effects, and random permanent environmental effects, respectively.

- (2) RENUMF90 program will be employed to sequentially renumber the animals.
- (3) The methodological procedures necessary for the successful execution of a GWAS experiment are depicted in Figure 1.
- (4) It is crucial to choose the population of interest in the first stage of GWAS, accounting for the ideal number of individuals to minimize the negative impacts on the study's accuracy and predictive capacity. Between 100 and 500 people can be included in GWAS sample sizes (Tam *et al.*, 2019; Long *et al.*, 2024).
- (5) In the subsequent phase, it is imperative to identify the associations between genotype and phenotype, necessitating that all individuals subjected to genotyping undergo phenotyping for particular traits that correspond with the objectives of the study (Tam *et al.*, 2019).
- (6) In this step, the whole blood samples are to be collected aseptically from the jugular vein of buffalo cows and bulls for the genomic DNA extraction employing the conventional phenol-

chloroform extraction procedure and the ethanol precipitation techniques (Sambrook *et al.*, 1989).

- (7) The extracted DNA is used to genotype buffalo animals using 90K array (<http://www.lgscr.it/ENG/index.html>) and the SNPs contained in this chip are based on buffalo sequencing data (Iamartino *et al.*, 2013). Numerous buffalo species have been utilized in GWAS, including the Brazilian buffalo (de Camargo and *et al.*, 2015), the Egyptian buffalo (El-Halawany *et al.*, 2017), and the Italian Mediterranean buffalo (Iamartino *et al.*, 2013). The initial number of SNPs is determined and distributed across the 24 autosomes and X-chromosome.
- (8) Before starting GWAS analysis in the third stage, it is crucial to assess the population structure (Marees *et al.*, 2018). The statistical connections between phenotypic characteristics and genetic markers are usually the main focus of GWAS procedure. Genotyping using the technique of SNP has been used in buffalo (Liu *et al.*, 2018, and Vohra *et al.*, 2021).
- (9) In the fourth phase, DNA molecular markers must be used to genotype the same group of phenotyped individuals. A quality control process is performed after genotyping to lessen the likelihood of discovering false positive or false negative associations. However, quality management of GWAS data requires at least three essential processes for each marker: a) Identifying the SNPs with a high number of missing genotypes by removing those with a low genotype call rate (the call rate is the percentage of study individuals for whom information are on a certain SNP, b) Finding and eliminating markers with extremely low minor allele frequencies (MAFs) suggests that there is insufficient statistical power to find meaningful relationships with the studied traits, c) SNPs that deviate significantly from Hardy-Weinberg equilibrium (HWE) are eliminated. To achieve a genotyping rate of 0.99, quality control is carried out using AffyPipe (Nicolazzi *et al.*, 2014) and PLINK V 1.9 (Purcell *et al.*, 2007, and Chang *et al.*, 2015).
- (10) After data filtration, the dataset involving the genotyped animals are used in genomic evaluations of the animals (Misztal *et al.*, 2018).
- (11) To assess the relevance of all available fixed factors and the animals' genetic evaluations, the mixed model approach included into the R software is used, taking into consideration the following parameters, model, and markers. (Luke, 2017):
 - a) Using their phenotypic data and the whole pedigree information, estimated breeding values (EBV), b) BLUPF90 software with a univariate animal model (Misztal *et al.*, 2018), c) SNPs

- with low minor allele frequencies (MAF<0.05), ambiguous physical locations, or missing genotypes per SNP (GENO>0.10) are excluded from the genomic research and d) Markers significantly ($P<0.001$) deviated from Hardy-Weinberg proportion are eliminated.
- (12) Utilizing the linear regression model included into the PLINK software, GWAS is carried out in the fifth phase (Purcell *et al.*, 2007). To regress the average daily deviations on the number of copies of the alleles, the PLINK-linear option will be employed as a covariate. The genome-wide complex trait analysis (GCTA) software version 1.25.3 will be used to determine the heritability of the average yield deviation (Yang *et al.*, 2011). To get a list of previously reported QTL for the traits under study, visit animal QTLdb, release 30 (Hu *et al.*, 2016; <http://www.animalgenome.org/QTLdb>).
 - (13) The sixth phase involves the annotation of the buffalo genome, biological processes, pathways, and functional study of the potential genes for economic traits (Deng *et al.*, 2019; Liu *et al.*, 2020; Pacheco *et al.*, 2020; Rehman *et al.*, 2021; Shao *et al.*, 2021; Vohra *et al.*, 2021, and Lázaro *et al.*, 2023).
 - (14) In the seventh step, estimating the linkage disequilibrium (LD) across the genome (Hayes *et al.*, 2003, and Lu *et al.*, 2020). Following the selection of the GWAS model, phenotypic and genotypic data may be analysed using the tools listed in this article to determine which alleles are linked to certain characteristics.
 - (15) Based on their genomic estimated breeding values (GEBV), the top buffaloes and bulls are selected in the eighth stage to be the parents of the upcoming generations (genomic selection). The GEBV will be calculated as the total effect of dense genetic markers across the entire genome. The QTL effects are estimated to be using phenotypic data from a large reference population.
 - (16) Displaying the GWAS findings and showing them in a Manhattan or QQ plot is the last step. The Bonferroni correction or the false discovery rates (FDR) are frequently used to illustrate the importance of markers (Kaler and Purcell, 2019). In addition to the tables that provide significant SNPs, minor allele frequency, sample size and the percentage of phenotypic variation attributable to the markers (R^2), Manhattan plots are frequently used to illustrate GWAS results.

Implementing single-step genome-wide association study (ss-GWAS):

Few genetic study approaches have been evaluated for assessing buffaloes for lactation, reproduction, growth, carcass, and semen traits. Wang *et al.* (2012) introduced previously a novel method called Single-Step Genomic Best Linear

Unbiased Prediction (ssGBLUP). This approach provides clear benefit and accuracy over typical GWAS in the simultaneous calculation of breeding values for both genotyped and non-genotyped animals by integrating genomic, pedigree, and phenotypic data into a single model. This, in turn, may increase the precision of predicting the animal genetic merit (Aguilar *et al.*, 2010, 2019). These input files are then used with GIBBS1F90, POSTGIBBSF90, BLUPF90, PREGSF90, and POSTGSF90 softwares to estimate variance components and conduct the ssGWAS analysis (Wang *et al.*, 2012; Aguilar *et al.*, 2019). The univariate animal model as defined before in this article will be applied using the inverse of numerator relationship matrix based on pedigree information (A^{-1}) and the inverse of genomic relationship matrix (G^{-1}) according to VanRaden (2008). In order to update the ssGBLUP solutions, the GEBVs obtained from ssGBLUP are transformed into marker effects and used in an iterative procedure (Wang *et al.*, 2012, and EL Nagar *et al.*, 2023).

Reliability of GWAS analyses:

The pedigree information, phenotypic data and the traditional estimated breeding values (EBV) must all be used to evaluate the reliabilities of GWAS analysis. The prediction accuracy will be used to assess both the GEBV's dependability and the correlation between EBV and GEBV (Moser *et al.*, 2009, and Zhang *et al.*, 2010). Liu *et al.* (2018) stated that the locations and markers impacting buffalo milk performance can be extrapolated to other populations. The accuracy of GEBV may be assessed by the degree of correlation between GEBV and EBV, which varied from 0.23 to 0.35 in the validation set (Gondro *et al.*, 2013). Generally, the reliability of GWAS could be improved in the following cases: 1) Adding more SNPs and expanding the population size (Goddard and Hayes, 2009; Zhang *et al.*, 2010, and Lu *et al.*, 2011), 2) The prediction accuracy is assessed using the correlation between EBV and GEBV and the dependability of GEBV (Moser *et al.*, 2009, and Zhang *et al.*, 2015), 3) The accuracy of genomic prediction may be improved by using more accessible phenotypes, genotypes, pedigree information, and genomic information in data sets (Pryce *et al.*, 2012), 4) Applying LR software (Legarra and Reverter, 2018) could improve the prediction accuracy and bias in GWAS, 5) Given that characteristics with higher heritability showed better GEBV reliability, using the trait's heritability might affect the accuracy of genomic predictions (Hayes *et al.*, 2009, and Zhang *et al.*, 2015), 6) Using ssGBLUP could be associated with an increase in prediction accuracy in buffalo, i.e. the accuracies in ssGBLUP were higher (0.75 ± 0.03) compared to the accuracies in classical BLUP (0.57 ± 0.03) (Cesarani *et al.*, 2021a,b).

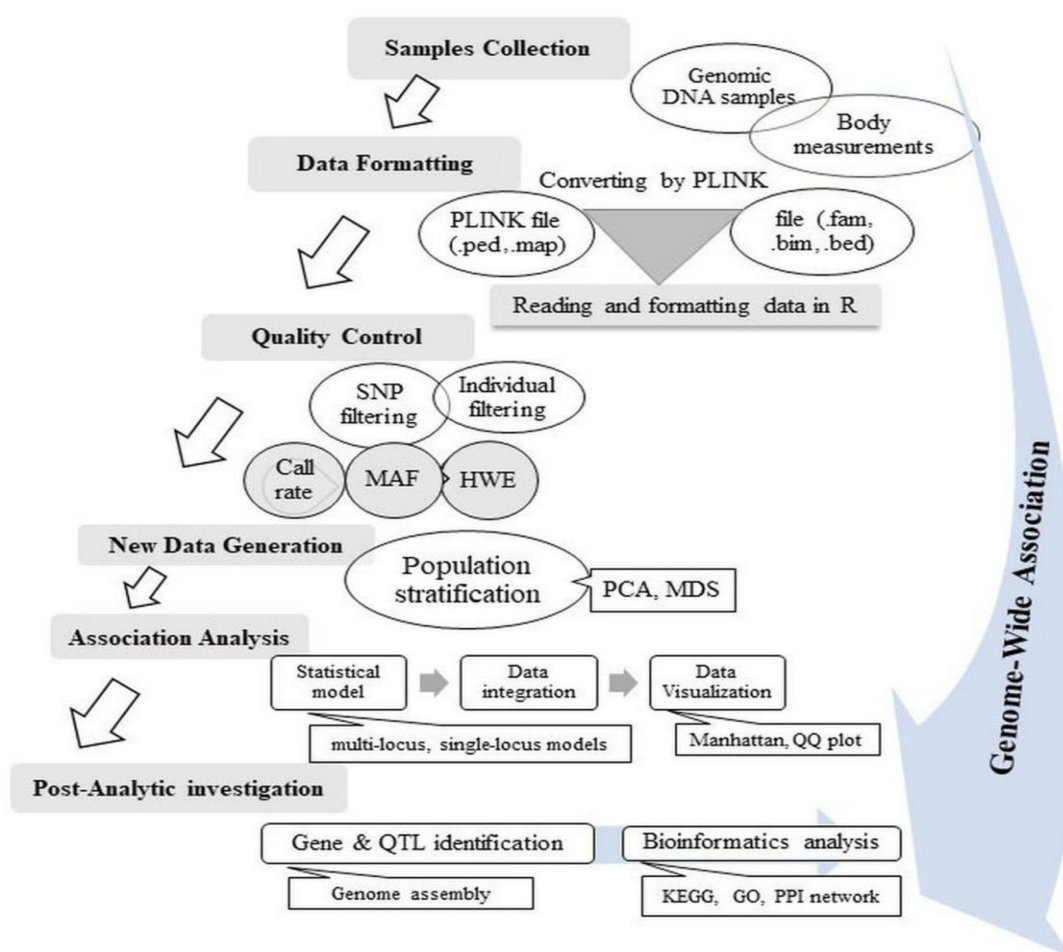


Figure 1. Flowchart illustrates the necessary procedures for carrying out a successful GWAS experiment on farm animals. (Source: Tan *et al.*, 2023, *Animal Research and One Health*, 1 (1), 56–77. <https://doi.org/10.1002/aro2.14>; Long *et al.*, 2024; *Animals* 14 (15), 2181; <https://doi.org/10.3390/ani14152181>).

The advantages of ssGBLUP over classical GWAS:

For milk, fat, and protein yields of the Italian Mediterranean buffalo, variance components, heritabilities, breeding values, BLUP and ssGBLUP were estimated. Model validation was then carried out to assess the prediction accuracy and biasness (Cesarani *et al.*, 2021a,b). Also, LR method was applied (Legarra and Reverter, 2018) in terms of correlation, accuracy, and bias statistics. As stated by Misztal *et al.* (2009), Yin *et al.* (2019), and Ma *et al.* (2023), the advantages of ssGBLUP relative to the classical GWAS could be summarized as follows: 1) ssGBLUP utilizes any phenotypic data for which marker effects or pedigree information is known, 2) It is applicable to any model. In comparison to pedigree-BLUP or GBLUP, it improves the accuracy of genomic evaluation (Onogi *et al.*, 2015; Matilainen *et al.*, 2016), 3) It may be applied to complicated datasets that contain both genotyped and non-genotyped animals (Aguilar *et al.*, 2019), 4) A combined relationship

matrix (H) is created by joining the genomic relationships matrix (G) and the population-based pedigree connection matrix (A). 5) This includes the ability to analyze numerous attributes and sophisticated models without the need for post-analysis processing (Koivula *et al.*, 2015, and Wang *et al.*, 2014), 6) It is an effective technique for pinpointing the genetic regions in charge of certain traits, and 7) It enables one-step genomic selection.

Plotting the Manhattan Plots from GWAS results:

As stated before, the National Centre for Biotechnology Information database (NCBI) will be used to search for the genes closely associated with production, reproduction and growth traits. It is preferable to show the results of GWAS using Manhattan plotting which will be obtained using R software (ver. 3.2.2, R Development core Team, 2013). R program is a compiled C or FORTRAN computer language program. It has become statistical programming language, and it is arguably the most widely used program for analysis of high-throughput genomic data. Additionally, GWAS

could be analyzed using PLINK software. For test-day yields of milk, fat, and protein as well as sperm concentration, Figures 2, 3, and 4 show some instances of Manhattan plots for additive genetic

variance in Egyptian buffalo explained by SNPs (Abdel-Shafy *et al.*, 2020a, and EL Nagar *et al.*, 2023, 2025).

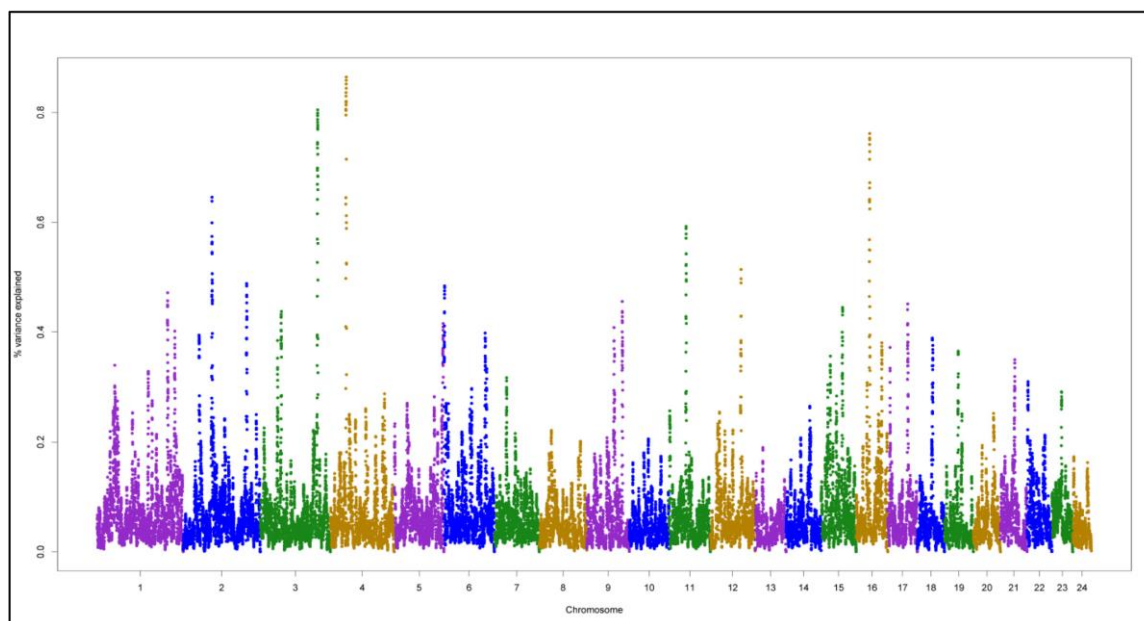


Figure 2. Manhattan plot illustrates how each of the 30 neighboring SNPs contributes to the daily milk yield of Egyptian buffalo. (Source: Abdel-Shafy *et al.*, 2020a, Livestock Science 234, 103977).

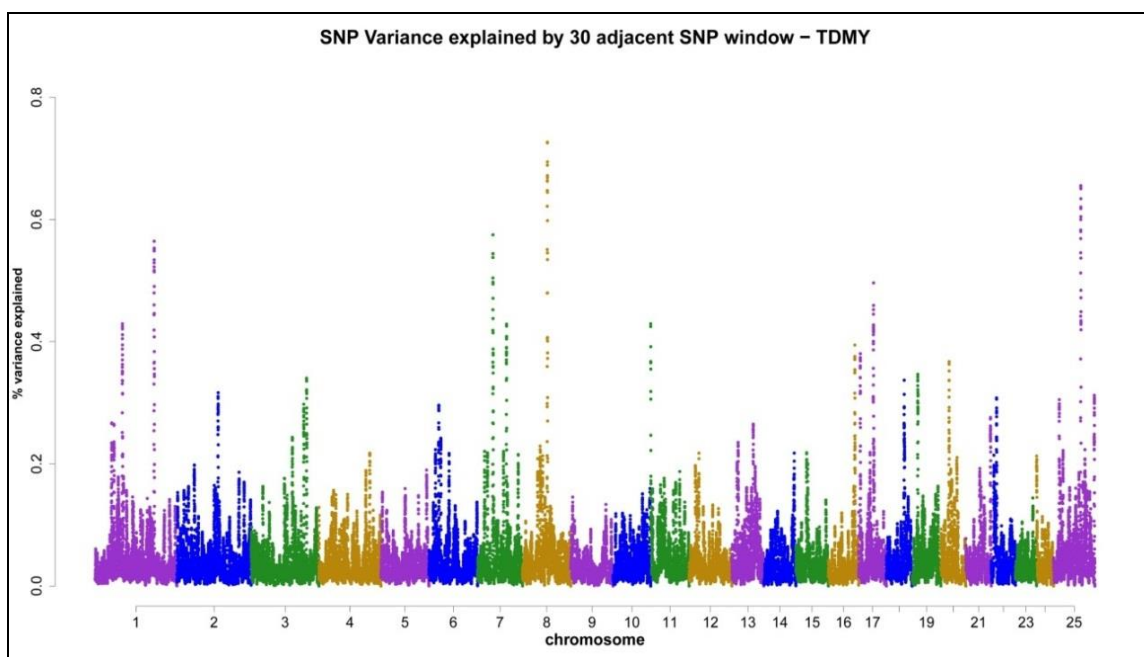


Figure 3. Manhattan plot uses both pedigree and genomic data to display the additive genetic variance explained by each of the 30 neighboring SNPs for test-day milk yield (TDMY) in Egyptian buffalo. (Source: EL Nagar *et al.*, 2025, under publication).

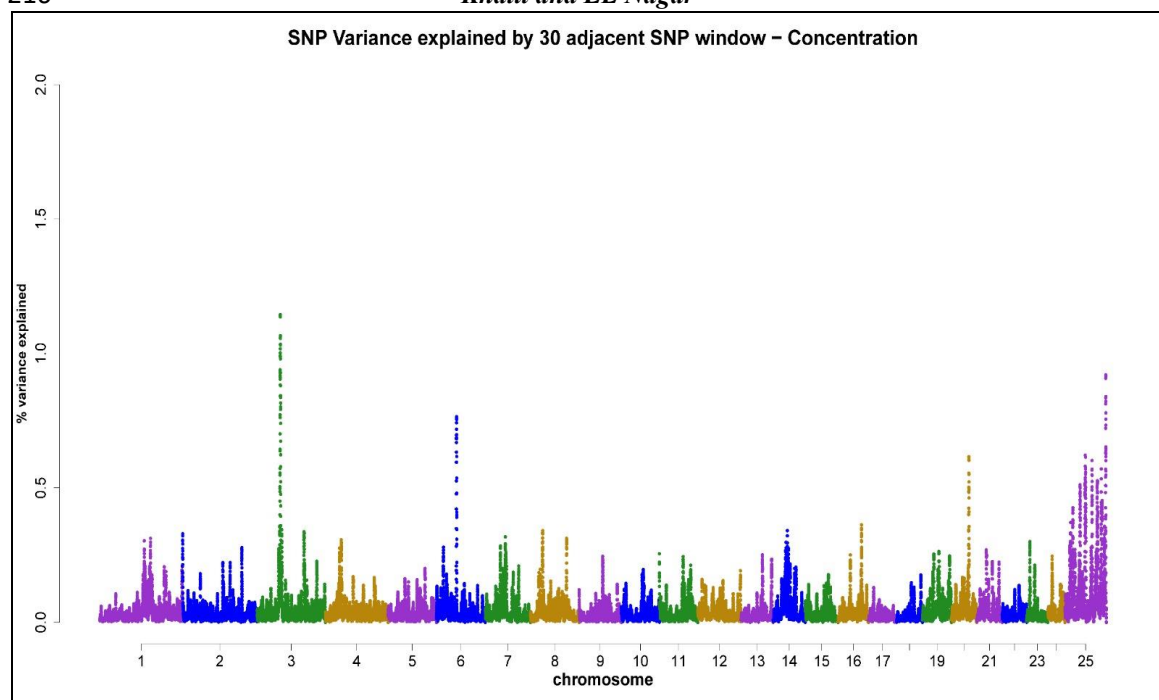


Figure 4.Manhattan plotting for sperm concentration in Egyptian buffalo bulls illustrates the additive genetic variance explained by each of the 30 neighbouring SNPs. (Source: ELNagar *et al.*, 2023, *Animals*, 13, 3758. <https://doi.org/10.3390/ani13243758>).

Putative candidate genes identified by GWAS analyses in the Egyptian buffalo studies:

The early genomic attempts conducted on buffalo in Egypt revealed that GWAS is crucial for determining the pertinent markers, causative mutations, genomic regions, and candidate genes linked to a number of economically significant breeding, growth, lactation, and semen traits (El-Halawany *et al.*, 2017; Abdel-Shafy *et al.*, 2020a,b, and EL Nagar *et al.*, 2023). In GWAS analyses applied on Egyptian buffalo, some candidate genes associated with milk production were detected on chromosome 1, 5, 6 and 27 by El-Halawany *et al.* (2017) using 42269 SNP for 96 animals. Also, Abdel-Shafy *et al.* (2020a,b) using 64169 SNP from 114 buffaloes in GWAS and ss-GWAS analyses found that the candidate genes associated with milk production were detected on chromosomes number 4, 5, 15 and 17. In ss-GWAS analyses for semen traits using 67282 SNP from 115 bulls with 84665 semen ejaculates, ELNagar *et al.* (2023) reported that five novel candidate genes (4 genes on X-chromosome and one gene on chromosome16) and four novel genes nominated as Melanoma-associated antigen D2 gene, Sodium/hydrogen exchanger 2-like gene, actin-related protein T1 gene, and cancer/testis antigen 47A-like gene were linked to spermatogenesis and male fertility. According to a recent study, the X chromosome accounts for 23.4% of the overall genetic variance observed in semen characteristics, making it the most significant chromosome. This indicates that it explains 4.2, 4.6, 5.2, 5.2, and 4.3% of the genetic

variance for the volume of semen ejaculate, sperm motility, sperm livability, sperm abnormalities, and sperm concentration, respectively. Similar to cattle, buffalo can undergo genomic selection to increase their lactation and fertility traits (Fortes *et al.*, 2020; Pacheco *et al.*, 2020, and Khan *et al.*, 2024).

Candidate and novel genes identified by GWAS analyses in non-Egyptian buffalo studies:

Earlier, Venturini *et al.* (2014) using bovine SNP chip in Brazilian buffalo reported that four candidate genes were identified for milk production traits and milk protein and fat percentages of 1018 individuals nominated as oestrogen-related receptor gamma (*ESRRG*), fragile histidine triad (*FHIT*), catenin delta 2 (*CTNND2*), and apolipo protein B (*APOB*). Additionally, de Camargo *et al.* (2015) using 90K SNP of GWAS analyses in Asian buffalo reported that the genes *KCTD8*, *ESRRG*, *TRNAY-AUA*, and *GPATCH2* were associated with fat %; the genes *SART3*, *ISCU*, *CMKLR1*, *WSCD2*, *MFNG*, *CARD10*, and *USP18* were associated with protein percentage; and the genes *BCL6*, *RTP2*, *SST*, and *PTGS2* were associated with milk production. Liu *et al.* (2018) in Italian Mediterranean buffalo identified several candidate genes through GWAS analyses for milk production namely *MFSD14A*, *SLC35A3*, *PALMD*, *RGS22* and *VPSI3B* genes. However, limited GWAS analyses have been performed in worldwide buffalo studies to detect the functional genes for milk production, milk compositions, reproduction and fertility traits (Deng *et al.*, 2019; Liu *et al.*, 2020; Pacheco *et al.*, 2020; Rehman *et al.*, 2021; Shao *et al.*, 2021, and

Lázaro *et al.*, 2023). GWAS was carried out for lactation and reproductive variables in Murrah buffalo because certain putative genomic areas were discovered to have a potential function in controlling milk yield and fertility (Vohra *et al.*, 2021). In this study, six SNPs on chromosomes 1, 6, and 9 were significantly associated with test-day milk yield, fat percentages, SNF percentages, 305 days' milk yield and lactation persistency, post-partum breeding interval, and age at sexual maturity. These genomic regions may prove beneficial, in the long term, for shaping future buffalo genetic improvement globally and in the short term, for early selection of high-yielding persistent buffaloes.

Genomic selection studies applied in buffalo:

The powerful marker-assisted selection, known as genomic selection (GS), is rarely applied in buffalo, though using genetic markers spans the entire genome effectively to ensure that all QTL are in linkage disequilibrium (LD). However, GS has been widely used in cattle, though it primarily focusses on carcass traits and milk production (Silva *et al.*, 2014, and Weller *et al.*, 2017). Moreover, there are very few genomic evaluation reports for production or reproduction characteristics in buffalo studies, or in other words the field is still in its infancy stage (de Araujo Neto *et al.*, 2020; Vohra *et al.*, 2021, and Lázaro *et al.*, 2023). The primary drawbacks of using genomic evaluation in buffalo include the absence of well-structured buffalo populations, as there are currently relatively limited numbers of buffaloes in each nation having both genotypic and phenotypic data. According to Liu *et al.* (2018), and Abdel-Shafy *et al.* (2020a,b), the most effective alternative approach to genomic selection would be a multi-breed genomic evaluation.

Highlighted conclusions concerning GWAS in buffalo:

GWAS approach in buffalo evaluation will be more common and efficient recently, leading to identifying additional genes related to complex traits in buffalo. The scope of genetic enhancement can be updated by applying GWAS technology to fully clarify the genomic principles underpinning complicated traits in buffalo breeding.

Future research on GWAS applications could be deepened in buffalo genetic breeding programs through 1) Creating new bioinformatics tools and data analysis methodologies, 2) Offering more thorough and efficient approaches and plans for buffalo genome exploring and enhancement, and 3) Enhancing the efficiency of breeding strategies for lactation, reproduction, semen quality and growth characteristics in buffalo using genomic selection programs. Since a larger sample size for GWAS analysis may help to improve the power of candidate gene detection and to obtain more

accuracy in GEBV to be used in genomic selection (Hayes *et al.*, 2009, and Van der Werf, 2013). A more accurate buffalo genomic map is necessarily required in order to identify the candidate genes affecting lactation, reproduction, growth, carcass, and semen traits.

Genomic selection can have a major impact on buffalo breeding programs and provide more accurate estimates for breeding value earlier in the life of breeding animals, giving more selection accuracy and allowing for lower generation interval (Van der Werf, 2013). Due to advancements in deep sequencing, SNP discovery methods, and throughput SNP genotyping on DNA chips, genomic selection is a viable strategy (Meyer and Tier, 2012). But, the current limitations of these technologies specific to the buffalo species (e.g., lack of reference genomes, costs, panel density) are not available to be discussed.

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إستخدام الارتباطات على مستوى الجينوم GWAS في التحسين الوراثي للصفات الإقتصادية في الجاموس: مقالة مرجعية

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يُعد برنامج إختبار النسل التقليدي المُطبق في الماشية بالدول المتقدمة أكثر تكلفة ويستغرق وقتًا أطول فيما لو تم تطبيقه على الجاموس في الدول النامية. في الوقت الحاضر، لا تزال دراسات الارتباط على مستوى الجينوم (GWAS) للصفات الإقتصادية في الجاموس محدودة جدًا مقارنةً بما هو موجود في ماشية اللبن. وتعتمد هذه الطريقة الجينومية على دراسة الجينوم الكامل للجاموس لتحديد الجينات المرتبطة بالصفات ذات الأهمية الاقتصادية. ومع توفر منصة SNP متوسطة الكثافة (90K array) خاصة بالجاموس حاليًا قد يكون ذلك مفيدًا لدمج المعلومات الجينومية مع البيانات المظهرية، وبالتالي تحسين دقة تقييم أفراد الجاموس وتسريع برامج التحسين الوراثي. يهدف هذا البحث المنهجية مع التطبيقات الخاصة بدراسات GWAS في الجاموس لتحقيق الآتي: (١) البحث في المواقع الإلكترونية عن الجينات المرشحة أن تكون مرتبطة بصفات الإدرار، التناسل، خصائص السائل المنوي، النمو وصفات الذبيحة، (٢) إستعراض كيفية تطبيق أسلوب التحليل باستخدام QTL ، GWAS على الجاموس ، (٣) التعريف بالرفائق SNP والواسمات الوراثية الضرورية لإجراء تحليلات GWAS، (٤) توضيح كيفية تداول قواعد البيانات الجينومية والبرمجيات الخاصة بتحليلات GWAS ، (٥) تطبيق طريقة GWAS ذات الخطوة الواحدة (Single-step GWAS) لتحليل بيانات GWAS ، (٦) عمل مخططات "مانهاتن" (Manhattan plots) بإستخدام نتائج GWAS لتحديد دور الجينات المرشحة في التحكم في الصفات الإقتصادية، (٧) إستعراض الجينات المرشحة التي تم تحديدها بواسطة تحليلات GWAS في دراسات الجاموس المصري وغير المصري، (٨) تقدير القيم التربوية الجينومية (GEBV) وتطبيق الإختخاب الجينومي (GS) ، (٩) عملياً، يمكن إستخدام القيم التربوية الجينومية المقدرة والمجدولة (GEBV) في الدراسات المصرية السابقة لإناث وطلائق الجاموس المصري المرباه بالقطعان التابعة لمعهد بحوث الإنتاج الحيواني (APRI) التي تم تقييمها للصفات الإقتصادية ضمن المشروعات البحثية الممولة من هيئة تمويل العلوم والتكنولوجيا والإبتكار STDF تمهيدا لإستخدامها كأباء في برامج التحسين الوراثي الجينومي بإستخدام نهج GWAS في الجاموس المصري.