

J. Dairy Sci. 106:9016–9025 https://doi.org/10.3168/jds.2023-23257

© 2023, The Authors. Published by Elsevier Inc. and Fass Inc. on behalf of the American Dairy Science Association[®]. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Genetic parameters for reproductive traits in the Italian Mediterranean buffalo using milk yield as a correlated trait

M. Gómez-Carpio,¹ A. Cesarani,^{2,3} G. Zullo,¹ R. Cimmino,¹ G. Neglia,⁴* G. Campanile,⁴ A. and S. Biffani⁵

¹Italian National Association of Buffalo Breeders, 81100 Caserta, Italy

²Dipartimento di Agraria, University of Sassari, 07100 Sassari, Italy

³Animal and Dairy Science Department, University of Georgia, Athens, GA 30602

⁴Department of Veterinary Medicine and Animal Production, Federico II University, 80137 Naples, Italy

⁵Consiglio Nazionale delle Ricerche (CNR), Istituto di biologia e biotecnologia agraria (IBBA), 20133 Milan, Italy

ABSTRACT

Until now, the genetic evaluation of the Italian Mediterranean Buffalo has been mainly focused on production traits. However, female fertility affects the efficiency of the dairy industry as it is essential to maintain the profitability of dairy farms. Indeed, the estimation of its genetic component is crucial for its improvement. In this study, 3 measures of buffalo's fertility were analyzed: the age at first calving (AFC), the interval between first and second calving (CIV1), and the interval between second and successive calvings (CIV2_12). Milk yield at 270 d (MY270) was used as a correlated trait. First, genetic parameters were estimated using 7.915 buffalo cows with first calving from 1991 to 2018, then breeding values were calculated from 236,087 buffalo cows. Genetic parameters were estimated by Bayesian inference fitting a multipletrait animal model using the GIBBS1F90 program, and BLUPF90 was used for estimation of breeding value. The heritability and repeatability estimates of fertility traits were low. The genetic correlations among fertility traits ranged from 0.10 (AFC-CIV1) to 0.92 (CIV1-CIV2_12). Genetic correlation between MY270 and fertility traits was unfavorable, ranging from 0.23 to 0.48. The results from this study can be used as a basis for the future genetic improvement of fertility traits in the Italian Mediterranean Buffaloes.

Key words: Mediterranean buffalo fertility, milk yield, heritability, genetic correlation

INTRODUCTION

The early breeding objective in the Italian Mediterranean buffalo (IMB) was mainly focused on improving production traits with a reduced or null emphasis on other functional traits. Indeed, the selection criteria most worldwide used in buffalo breeding were, and still are, those associated with productive features (Aspilcueta-Borquis et al., 2010; Gupta et al., 2015; Costa et al., 2020). In this species, even the most recent studies on the genomic selection were oriented to the production traits (Iamartino et al., 2017; Cesarani et al., 2021). Furthermore, excellent milk quality and its high price (mainly in Italy) contributed to a breeding selection focusing more on milk production than on reproductive traits (Shao et al., 2021). Nevertheless, reproductive efficiency is considered an important factor that affects total milk production, as it is associated with calving performances. Consequently, in the last years selection for fertility has become an important breeding goal in different livestock species, such as beef and dairy cattle (Liu et al., 2008; Robinson, 2008; VanRaden et al., 2014).

In general, low reproductive capacity or infertility problems require additional inseminations, more veterinary attention, and hormonal treatments, which consequently alter the current and subsequent lactations (Boichard, 1990). Also, additional costs are raised due to culling and replacing animals with fertility problems (Roxström and Strandberg, 2002). Therefore, improving livestock fertility is the best option to reduce management costs and increase farm profits (Dekkers, 1991). One of the main problems in genetically improving fertility in livestock, is to identify and use the most informative trait. Among the most common and used reproductive traits are the age at first calving (AFC), which actually represents the starting point of a young female buffalo productive career, the calving interval (CIV), the number of inseminations for conception and the pregnancy rate (Tonhati et al., 2000; Shao et al., 2021).

The selection of animals for sexual precocity results in the reduction of the AFC, enabling higher profits and faster recovery of capital invested because of the reduction of herd maintenance costs. From a genetic

Received January 12, 2023.

Accepted June 16, 2023.

^{*}Corresponding author: neglia@unina.it

standpoint, this selection can result in a shorter interval between generations and a faster rate of genetic progress per unit of time obtained as a response to selection (Fernandes et al., 2016).

Unfortunately, the genetic progress of reproductive traits is slower than productive ones, because they are quite complex and affected by several factors, especially nongenetic. Such a situation leads to low heritability estimates of buffalo reproductive traits (Shao et al., 2021). An additional constraint is observed in Italy, where the breeding season can be voluntarily postponed by the farmer. Indeed, most of the Italian market demands for buffalo milk, to be used for the production of the Mozzarella di Bufala Campana cheese, occur in the period of the year when calving is less frequent (i.e., spring and summer), due to negative photoperiod features of the species (D'Occhio et al., 2020). Therefore, a specific strategy, generally called "out-ofbreeding-season-mating" technique, has been adopted to reverse the calving season in IMB (Zicarelli, 1997). This technique relies on using artificial insemination or natural mating between March and September to have the calving period between January and August of the following year. However, this approach is prone to reproductive disorders and, therefore, a gradual application using only a batch of the available females (mainly primiparous) is generally suggested to mitigate fertility loss within the herd.

Many studies investigated the genetic variation of reproductive traits in different buffalo populations, showing a low heritability, mainly for AFC and CIV, and a negative (i.e., unfavorable) correlation with productive traits (Kumar et al., 2015; Barros et al., 2016; Rathod et al., 2018). Moreover, the heritability of the same trait varies greatly among different breeds, suggesting the need to evaluate each breed separately (Cassiano et al., 2004).

For this reason, the aim of the present study was first to estimate the genetic parameters and then breeding values and genetic trends of 3 reproductive traits in the IMB.

MATERIALS AND METHODS

Ethics Statement

Animal welfare and use committee approval was not needed for this study as data sets were obtained from pre-existing databases based on routine animal recording procedures.

Data

Data for the present study were provided by the National Association of Italian Buffalo Breeders (ANASB) and regarded reproductive, productive, and pedigree information. The initial data set included a total of 6,576,041 milk test day samples collected between 1982 and 2019 from 262,254 buffalo cows. Additional available information were: birth-date, calving date, herd of calving, and milk yield adjusted to 270 d (MY270). The editing criteria to exclude extreme values included the following restrictions: AFC <23 and >47 mo, CIV <350 and >800 d, and birth orders >12.

The following traits were considered in this study as measures of buffalo's fertility: AFC, the first CIV (i.e., the interval between first and second calving, **CIV1**) and the interval between the second and subsequent calvings (**CIV2_12**), with a maximum number of calving equal to 12 (i.e., **CIV2_3**; **CIV3_4**;

... CIV11_12). All traits were expressed in days. Four calving and birth seasons were defined: spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February). In addition, herd size was defined in 5 classes, 1: <100, 2: \geq 100 and <200, 3: \geq 200 and <300, 4: \geq 300 and <400, and 5: \geq 400 animals. Additionally, MY270—calculated according to the guidelines of the International Committee for Animal Recording (ICAR) for Buffalo Milk Recording (ICAR, 2021)—was also included as a correlated trait. In general, the inclusion of MY270 has 2 main objectives: first, to take into account its genetic correlation with fertility; and second, to correct for the bias due to voluntary culling of low-producing individuals.

Fertility traits are complex traits, mainly because they are strongly influenced by nongenetic aspects. For this reason, we have set a very rigorous editing of the data for the estimation of the genetic parameters (for example, only buffaloes with sire and dam tested with DNA). Once the parameters have been estimated, we have calculated the genetic index for the whole population. Therefore, after editing 2 different data sets were created: a first one used for the estimation of variance components (VCE) and a second one for breeding value estimation and calculation of genetic progress. The former contained records of milk yield (25,375), AFC and first CIV1 (7,915) and successive $CIV2_{12}$ (21,193), collected from 7,915 buffalo cows with first calving from 1991 to 2018 in 258 herds. The latter included 719,797 observations corresponding to 236,087 buffaloes. In total, the pedigree included 22,456 animals for VCE and 650,277 for EBV.

Models and Genetic Parameter Estimation

The following multivariate animal model was fitted to estimate VCE and EBV for fertility and milk yield traits in the IMB breed: • AFC, CI1:

$$\mathbf{y} = \mu + \mathbf{X}\mathbf{b} + \mathbf{Q}\mathbf{h} + \mathbf{Z}\mathbf{a} + \mathbf{e};$$

CI2_12, MY270:

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{X}\mathbf{b} + \mathbf{Q}\mathbf{h} + \mathbf{Z}\mathbf{a} + \mathbf{W}\mathbf{p}\mathbf{e} + \mathbf{e},$$

where \mathbf{y} is the vector of observations of each trait (AFC, CIV1, CIV2_12, MY270); μ is the overall mean; **b** is the vector of fixed effects, which included the effect of the combination of year and season of birth (105 levels), herd size (5 levels), daughter of artificial insemination (2 levels: yes or no), year and month of calving (273 levels), and parity for the CIV2_12, MY270 (1, 2, 1)3, ... 12); **h** is the vector of random herd effect; **a** is the vector of random animal effect; **pe** is the vector of random permanent environmental effect; e is the vector of residual effect; and X, Q, Z, and W are incidence matrices relating records to fixed, herd, animal, and permanent environmental effects, respectively.

The GIBBS1F90 program (Misztal et al., 2002) was used for estimating (co)variance components using Gibbs sampling. For the analysis, 500,000 samples (saving every 50 samples after discarding a burn-in of 100,000 iterations) were drawn. Convergence was determined from a visual inspection of trace plots. The POSTGIBBSF90 module of BLUPF90 family of programs (Misztal et al., 2002) was used to extract the posterior mean as a point estimate of (co)variance components and related parameters. Lower and upper bounds of the 95% highest posterior probability density regions (HPD) for heritability and additive genetic correlations were estimated from the Gibbs samples. and the EBV were obtained using BLUPF90 (Misztal et al., 2018). Data preparation, editing, and all statistical analyses were performed using the R programming environment v.3.6.1 (R Core Team, 2019).

The heritability (h^2) was computed as

• AFC, CIV1:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_h^2 + \sigma_e^2};$$

• CIV2_12, MY270:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_h^2 + \sigma_{pe}^2 + \sigma_e^2},$$

where σ_a^2 is the additive genetic variance, σ_h^2 is the herd variance, σ_{pe}^2 is the permanent variance, and σ_e^2 is the residual variance.

Intraherd heritability (h_h^2) was calculated as

AFC, CIV1:

$$h_h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2};$$

CIV2_12, MY270:

$$h_h^2 = rac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

The repeatability (r) was calculated only for CIV2_12 and MY270, as

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_h^2 + \sigma_{pe}^2 + \sigma_e^2}$$

Realized genetic gains for the 3 fertility traits were estimated from the linear regressions of the buffalo cows' EBV for each trait on their birth dates. Buffalo cows with phenotypic data and born from 1985 to 2016 were used. Additionally, to identify possible breakpoints in the realized genetic trends, a piecewise regression model was also fitted to the same data using results from the linear regression. The function segmented of the R package segmented (Muggeo, 2003, 2008, 2017) was used.

RESULTS

Descriptive Statistics

Descriptive statistics for fertility traits in IMB and their phenotypic trends by year of birth are shown in Table 1 and Figure 1, respectively. The AFC ranged between 705 and 1,425 d, with an average of 1,024 d \pm 138. The coefficient of variation was low (13%). Phenotypically we can observe a steady decline of AFC until 2005 followed by a new increase and again a decrease.

As expected because of the editing criteria, parameters for CIV1 and CIV2_12 were similar and ranged between 350 and 800 d. Their averages varied from 473 $d \pm 100$ (for CIV1) to 452 $d \pm 98$ (for CIV2_12). The coefficient of variation (CV; $\sim 21\%$) was slightly higher than AFC, but the phenotypic trend (Figure 1) is more

9018

Gómez-Carpio et al.: REPRODUCTIVE TRAITS IN ITALIAN MEDITERRANEAN BUFFALO

Trait^1	Mean	SD	Minimum	Maximum	CV (%)
AFC (d) CIV1 (d) CIV2_12 (d) MY270 (kg)	$1,024 \\ 473 \\ 452 \\ 2,736$	$ \begin{array}{r} 138 \\ 100 \\ 98 \\ 582 \end{array} $	$705 \\ 350 \\ 350 \\ 1,301$	1,425 800 800 5,806	13 21 22 21

Table 1. Descriptive statistics for fertility and milk yield traits in the Italian Mediterranean Buffalo (IMB)

 ^{1}AFC = age at first calving; CIV1 = interval between first and second calving; CIV2_12 = interval between second and remaining calving; MY270 = 270-d adjusted milk yield.

stable and suggests a slow increase across years. The large drop in the last years observed for all traits is related to data censoring (i.e., a buffalo cow born in 2016 did not have a second calving, yet).

Across the observed period the average of the MY270 was 2,736 kg, ranging between 1,301 and 5,806 kg. Milk yield increased constantly until 2011 and then experienced a drop until 2015, followed by a further recovery.

Genetic Parameters

Posterior means estimates of variance components (additive, herd, permanent environmental, and residual), heritabilities (h^2) and repeatabilities (r) for the observed traits are reported in Table 2. The estimates of the additive genetic variance for AFC and CIV1 were greater than those observed in CIV2_12, which suggests a larger environmental variability for the latter trait. The estimates of heritabilities had a low magnitude and ranged from 0.03 for CIV2_12 to 0.09 for AFC. Intraherd heritability had a similar pattern, ranging from a minimum of 0.03 for CIV2_12 to a maximum of 0.10 in AFC. The repeatability for CIV2_12 showed low estimated value (0.06), a value of the same magnitude as their corresponding heritability. Heritability for MY270 was 0.27.

The estimates of genetic correlations and their HPD are shown in Table 3. The relationship among fertility traits were all positive ranging from 0.10 (AFC-CIV1) whose HPD included 0, to 0.41 (AFC-CIV2_12). Esti-



Figure 1. Phenotypic trend for fertility traits and milk yield in the Italian Mediterranean buffalo. $AFC = age at first calving; CIV1 = interval between first and second calving; CIV2_12 = interval between second and remaining calving.$

9019

the Italian

п.

given in parentheses] intervals) of fertility traits

genetic parameters (lower and upper bounds of the 95% highest probability density [HPD,

of

Estimates

ä

Table

mated genetic correlation between CIV was 0.92. The relationship between reproduction and milk production was also positive ranging from 0.23 for MY270-AFC to 0.48 for MY270-CIV2_12. However, a positive relationship should be considered as unfavorable because it means not only a postponed beginning of the reproduction activity but also longer intervals between calvings.

Genetic Trends

The genetic trends for the 3 fertility traits estimated from buffalo cows born between 1985 and 2016 are in Figure 2. Results from piecewise regression are in Table 4. A moderate increase was observed in the genetic trend for AFC. The piecewise regression identified one breakpoint between 2000 and 2001. Before 2000 the genetic increase per year was 0.026, which decreased to approximately 0 (b = 0.007) afterward. The genetic trends for CIV1 and CIV2_12 were quite similar, and the piecewise regression identified 3 and 4 breakpoints, respectively. Until 1991, both CIV1 and CIV2_12 decreased yearly by -0.128 and -0.077, respectively. Afterward, CIV1 showed a negative or approximately null trend (b = -0.007) until 2011 while CIV2_12 had a positive yearly increase (i.e., unfavorable) of 0.01 until 2008 and of 0.05 from 2009 until 2011. Finally, since 2011 both traits showed a larger yearly increase than before, being 0.106 and 0.132 for CIV1 and CIV2_12, respectively.

DISCUSSION

Bubalus bubalis, the water buffalo, is considered one of the most rustic domestic species, able to survive in extreme wet or swampy conditions (Minervino et al., 2020; Neglia et al., 2020; Gómez et al., 2021). However, it also represents an important financial asset in both developing and developed countries (e.g., the production in Southern Italy of Mozzarella di Bufala Campana Protected Designation of Origin cheese), which actually requires sustainable and economically efficient production systems.

Reproductive traits are economically important for sustainable food production. Indeed, low reproductive capacity or infertility (e.g., an extended duration between 2 calvings, additional inseminations, veterinary interventions, and hormonal treatments) increase costs as well as the probability of an animal being culled or replaced (Boichard, 1990; Roxström and Strandberg, 2002; Shao et al., 2021).

One effective technology that produces permanent and cumulative changes in performance and whose power of long-term improvement has been well documented in various livestock species is genetic improve-

Trait	σ_a^2	σ^2_{pe}	$\sigma^2_{ m random}$	$\sigma_{ m r}^2$	h^2	h^2 (intraherd)	r
AFC	532.61		688.17	4,757.06	0.09	0.10	
	(320.26 - 758.55)		(442.92 - 971.97)	(4,519.14 - 5,001.49)	(0.05-0.13)	(0.07 - 0.13)	
CIV1	749.45		653.09	8,533.88	0.08	0.08	
	(481.99 - 1, 021.31)		(377.66 - 947.64)	(8, 166.45 - 8, 893.13)	(0.05 - 0.10)	(0.06 - 0.10)	
$CIV2_12$	262.32	291.63	430.51	8,355.80	0.03	0.03	0.06
	(186.84 - 339.67)	(162.71 - 432.12)	(271.35 - 593.76)	(8, 149.10 - 8, 548.36)	(0.02 - 0.04)	(0.02 - 0.04)	(0.04 - 0.08)
MY270	86,979.31	37,566.86	73,025.81	121,893.51	0.27	0.35	0.39
	(75, 857.79 - 98, 628.65)	(28, 874.68 - 46, 015.43)	(54, 947.83 - 92, 572.21)	(119, 335.60 - 124, 580)	(0.24 - 0.31)	(0.34 - 0.37)	(0.38 - 0.40)

repeatability

r =

variance.

= residual

= herd variance. $\sigma_{\rm r}^2$

 $\sigma^2_{\rm random}$

environmental variance.

permanent

variance. σ_{pe}^2

genetic

additive

Table 3. Estimates (posterior mean and 95% highest probability density [HPD] intervals) of genetic correlations among fertility traits and milk yield in the Italian Mediterranean buffalo $(IMB)^1$

Trait	Mean	HPD interval (95%)		
AFC-CIV1	0.10	-0.109	0.304	
AFC-CIV2_12 AFC-MY270	$0.41 \\ 0.23$	$0.230 \\ 0.127$	$0.588 \\ 0.340$	
CIV1-CIV2_12 CIV1-MY270	$0.92 \\ 0.28$	$0.856 \\ 0.161$	$0.964 \\ 0.398$	
CIV2_12-MY270	0.48	0.368	0.596	

 ^{1}AFC = age at first calving; CIV1 = interval between first and second calving; CIV2_12 = interval between second and remaining calving; MY270 = 270-d adjusted milk yield.

ment (Pryce et al., 2000). However, its efficacy depends on how much of the observed phenotypic variation is due to genetic variation (i.e., narrow-sense heritability), eventually affecting the rate of genetic improvement. Over the past 20 years, several studies were conducted to estimate the heritability of different reproductive traits, but most of them were in cattle (Ma et al., 2019; Cesarani et al., 2020; Martinez-Castillero et al., 2020).

Moreover, these traits are largely influenced by environmental factors, such as the reproductive management of the herd, whereby the models fitted in our study included the herd as a random effect. The inclusion of a random effect had a dual purpose: not only a better management of information coming from small groups of contemporaries (Visscher and Goddard, 1993) but also to model the nongenetic covariance between individuals producing in the same farm and that can therefore share common farmer's managerial choices (i.e., synchronization, voluntary waiting period, and so on). Indeed, contemporary groups are generally used to identify groups of same-sex animals, born or producing in the same period, or raised under the same conditions, or who have received the same managerial care (Schaeffer, 2018). In this way, the model should better reflect any characteristics that influence all responses for that particular farm (Biffani et al., 2020). In the present study, the percentage of change observed for fertility traits due to the herd ranged from 5% to 12%.

The h^2 value estimated for AFC in the present study was similar to those from buffalo populations in Brazil (0.07), Egypt (0.11) and India (0.14; de Oliveira Seno et al., 2007; El-Bramony, 2011; Gupta et al., 2015). However, they were lower than those estimated in other studies on the Murrah breed, where heritabilities ranged from 0.17 to 0.28 (Tonhati et al., 2000; de Camargo et al., 2015; Kumar et al., 2015). Our heritability for CIV1 was slightly higher than those reported by Cassiano et al. (2004), Fernandes et al. (2016), and de Araujo Neto et al. (2020), but lower compared with what was reported by de Oliveira Seno et al. (2007) in the Murrah breed in Brazil (0.14). In our study we also evaluated CIV other

than the first (i.e., CIV2_12), whose estimate was low (0.03), but similar to what reported by Cassiano et al. (2004), in the Carabao, Jafarabadi, and Mediterranean buffalo breeds. Slightly higher heritabilities, with values ranging from 0.05 to 0.10, were published in other studies (Tonhati et al., 2000; Morammazi et al., 2007; Barros et al., 2016). In contrast, high heritability values were reported for CIV2_12 in the Indian Murrah (0.23; Gupta et al., 2015) and Suri (0.55; Rathod et al., 2015)2018) breeds. Heritability estimates for fertility-related traits are usually obtained from field data that may be biased by a breeder's attitude. For example, calving interval measures frequently omit dams discarded for low production or reproductive problems, a strategy that can result in a reduction of additive genetic variation (Malhado et al., 2013). Even in the same breed, the different populations showed variations among estimated parameters, which may be related to different management and performance that the fitted models were not able to capture or control (Shao et al., 2021).

This suggestion is also supported by results for repeatability of CIV2_12, which was extremely low (0.06). Repeatability is a statistic that describes the degree to which variation within individuals contributes to total variation in a population and may be set as an upper limit on heritability (Boake, 1989). In our study we observed that at the most 6% of the phenotypic differences among buffalo cows were dependent on additive genetic and permanent environmental factors.

Genetic correlations between AFC and CIV were low to moderate (0.10 and 0.41) and highest between CIV (0.92). These results suggest that selection for earlier AFC would have a favorable effect on reduction of calving interval. Therefore, direct selection for either of these traits may result in genetic gains in both. Similar results were reported in both buffaloes and cattle (de Oliveira Seno et al., 2007; Berry and Evans, 2014; Barros et al., 2016), while negative correlations have been reported in the Egyptian buffalo, Nellore, and Brahman breeds (Mercadante et al., 2000; El-Bramony, 2011; Cavani et al., 2015). However, using AFC or CIV as selection criteria should also depend on additional justification. Indeed, can a reduction in CIV be an advantage? For example, in US dairy goats, selecting for shorter CIV decreased the productive life of females at 72 mo by 8.28 d per generation (Castañeda-Bustos et al., 2014). A similar attitude might not be beneficial in buffaloes, first because calving for the first time too early might jeopardize heifer development, and then because productive life might be reduced. An optimal solution would be the use of an aggregate selection index that includes both traits and whose relative emphasis should depend on actual phenotypic and genetic population parameters (e.g., if the average AFC in





Figure 2. Genetic trends for fertility traits in the Italian Mediterranean buffalo. Results from a piecewise regression model are also shown (b coefficients). AFC = age at first calving; CIV1 = interval between first and second calving; CIV2_12 = interval between second and remaining calving.

the active population is considerably high, a possible selection objective would be to decrease it). In spite of low heritability, the genetic relationship between CIV1 and CIV2_12 was very high. This is not an unexpected result because genetic correlations represent the proportion of overlapping genetic effects; that is, 2 traits

could both be highly heritable but not be genetically correlated or have low heritability and be completely correlated (Lukowski et al., 2017).

An additional and important result from our study regards the relationship between fertility and production. In dairy cattle a plethora of studies have not only

9022

Gómez-Carpio et al.: REPRODUCTIVE TRAITS IN ITALIAN MEDITERRANEAN BUFFALO

Trait	Breakpoint	В	SE	CI95L	CI95U
AFC	1985-2000	0.026	0.009	0.008	0.044
	2001 - 2016	0.007	0.005	-0.003	0.017
CIV1	1985 - 1991	-0.128	0.047	-0.221	-0.035
	1992 - 2010	-0.007	0.006	-0.018	0.004
	2011 - 2016	0.106	0.033	0.042	0.171
CIV2_12	1985 - 1991	-0.077	0.031	-0.139	-0.016
	1992 - 2006	0.010	0.005	0.000	0.020
	2007 - 2011	0.051	0.032	-0.011	0.113
	2012-2016	0.132	0.031	0.072	0.192

Table 4. Realized genetic trends and results from the piecewise regression model between EBV and birth year¹

 ^{1}AFC = age at first calving; CIV1 = interval between first and second calving; CIV2_12 = interval between second and remaining calving. B = slope of linear fit. CI95L = lower bound of 95% CI. CI95U = upper bound of 95% CI.

demonstrated the unfavorable correlation between milk production traits and female fertility (Lucy, 2019) but also the problem that approximately all these traits (fertility, morphology, etc.) are biased with respect to milk production. Indeed, voluntary culling due to low production or preferential treatment of superior milk producers (e.g., reinsemination) are 2 examples of such an issue (Weller, 1989; Biffani et al., 2005). Our genetic correlations between milk production and fertility traits were 0.23, 0.28 and 0.48 for AFC, CIV1 and CIV2_12, respectively. Those results confirm the unfavorable relationship and were not entirely unexpected. Indeed, as Negrini et al. (2021) pointed out, for a long time the main breeding goal of the IMB has been milk yield and kg of mozzarella and only recently it has been changed toward a more sustainable and welfare-oriented breeding goal (Biffani et al., 2021). Similar results were reported in the Murrah buffaloes from Brazil (Tonhati et al., 2000; Malhado et al., 2013; Barros et al., 2016) and in the Egyptian buffalo, where the first and second lactation showed a positive correlation with milk yield (\mathbf{MY}) , while a negative correlation was found in the third lactation (El-Bramony, 2011). In contrast, Malhado et al. (2009) showed a genetic correlation of -0.25 between MY and CIV traits in crossbred buffaloes in Brazil.

Even if these results confirm that selection for MY can affect genetic and phenotypic traits related to fertility, a recent paper in Israeli dairy cattle by Weller et al. (2022) showed how improving both traits is possible.

The realized genetic trend for AFC has been extremely moderate until 1999 and close to zero since 2000. Those results, even if different from those found by Fernandes et al. (2016) and Tonhati et al. (2000), are quite expected not only because the heritability of this trait is quite low (0.10) but also because it has never been included or even considered in any breeding schemes. Similar results were observed for both CIV1 and CIV2_12 even if the magnitude of the yearly change was slightly larger than AFC. Calving traits, as well as AFC, have never been included in any IMB breeding objective, and this means that the observed realized genetic trends are a good example of correlated response to the selection for MY. This important result confirms that a sustainable breeding objective must consider the unfavorable genetic response in fertility traits when selecting only for MY. In this sense, fertility must be included among the breeding objectives as already happened in dairy cattle (Miglior et al., 2017).

CONCLUSIONS

Our study was the first one to use a multitrait approach to estimate genetic parameters for fertility traits in the IMB. Moreover, we have also included MY as a correlated trait. Results have confirmed that genetic improvement of those traits is not easy because the genetic component is low and nongenetic factors play a major role. However, we observed a genetic trend for all the investigated traits which was basically due to the unfavorable genetic relationship between fertility and MY. This is something well established in dairy cattle, but now it must also be considered in buffaloes. This is not a trivial matter because MY is still the main objective for IMB farmers and producers. Therefore, selection should be based on an adequate and economically sustainable breeding objective. An additional way of improving fertility in the medium and short term might be the use of genomics. Again, positive results from dairy cattle have paved the way for its application in other species, and preliminary investigations in IMB are on the way.

ACKNOWLEDGMENTS

This research was funded by Italian Ministry of Agriculture (MIPAAF–DISR 07; Rome, Italy)–Programma di Sviluppo Rurale Nazionale 2014/2020. Caratterizzazione delle risorse genetiche animali di interesse zootecnico e salvaguardia della biodiversità. Sottomisura: 10.2–Sostegno per la conservazione, l'uso e lo sviluppo sostenibili delle risorse genetiche in agricoltura. Project: "Bufala Mediterranea Italiana—Tecnologie innovative per il miglioramento Genetico—BIG" Prot. N. 0215513 11/05/2021. CUP ANASB: J29J21003720005; CUP UNINA: J69J21003020005. The authors have not stated any conflicts of interest.

REFERENCES

- Aspilcueta-Borquis, R. R., F. R. Araujo Neto, F. Baldi, A. B. Bignardi, L. G. Albuquerque, and H. Tonhati. 2010. Genetic parameters for buffalo milk yield and milk quality traits using Bayesian inference. J. Dairy Sci. 93:2195–2201. https://doi.org/10.3168/jds .2009-2621.
- Barros, C. D. C., R. R. Aspilcueta-Borquis, A. B. Fraga, and H. Tonhati. 2016. Genetic parameter estimates for production and reproduction traits in dairy buffaloes. Rev. Caatinga 29:216–221. https: //doi.org/10.1590/1983-21252016v29n125rc.
- Berry, D. P., and R. D. Evans. 2014. Genetics of reproductive performance in seasonal calving beef cows and its association with performance traits. J. Anim. Sci. 92:1412–1422. https://doi.org/ 10.2527/jas.2013-6723.
- Biffani, S., M. Gómez, and A. Cesarani. 2021. Pitfalls and opportunities of genetic and genomic evaluation in the Buffalo species: Experiences from Italy. Interbull Bull. 56:2021.
- Biffani, S., M. Marusi, F. Biscarini, and F. Canavesi. 2005. Developing a genetic evaluation for fertility using angularity and milk yield as correlated traits. Interbull Bull. 33:2005.
- Biffani, S., F. Tiezzi, P. Fresi, A. Stella, and G. Minozzi. 2020. Genetic parameters of weeping teats in Italian Saanen and Alpine dairy goats and their relationship with milk production and somatic cell score. J. Dairy Sci. 103:9167–9176. https://doi.org/10.3168/ jds.2020-18175.
- Boake, C. R. B. 1989. Repeatability: Its role in evolutionary studies of mating behavior. Evol. Ecol. 3:173–182. https://doi.org/10.1007/ BF02270919.
- Boichard, D. 1990. Estimation of the economic value of conception rate in dairy cattle. Livest. Prod. Sci. 24:187–204. https://doi.org/ 10.1016/0301-6226(90)90001-M.
- Cassiano, L. A. P., A. S. Mariante, C. McManus, J. R. F. Marques, and N. A. Costa. 2004. Parâmetros genéticos das características produtivas e reprodutivas de búfalos na Amazônia brasileira. Pesqui. Agropecu. Bras. 39:451–457. https://doi.org/10.1590/S0100 -204X2004000500007.
- Castañeda-Bustos, V. J., H. H. Montaldo, G. Torres-Hernández, S. Pérez-Elizalde, M. Valencia-Posadas, O. Hernández-Mendo, and L. Shepard. 2014. Estimation of genetic parameters for productive life, reproduction, and milk-production traits in US dairy goats. J. Dairy Sci. 97:2462–2473. https://doi.org/10.3168/jds.2013-7503.
- Cavani, L., D. A. Garcia, L. O. D. Carreño, R. K. Ono, M. P. Pires, M. M. Farah, H. T. Ventura, D. D. Millen, and R. Fonseca. 2015. Estimates of genetic parameters for reproductive traits in Brahman cattle breed. J. Anim. Sci. 93:3287–3291. https://doi.org/10 .2527/jas.2015-8970.
- Cesarani, A., S. Biffani, A. Garcia, D. Lourenco, G. Bertolini, G. Neglia, I. Misztal, and N. P. P. Macciotta. 2021. Genomic investigation of milk production in Italian buffalo. Ital. J. Anim. Sci. 20:539– 547. https://doi.org/10.1080/1828051X.2021.1902404.
- Cesarani, A., G. Gaspa, Y. Masuda, L. Degano, D. Vicario, D. A. L. Lourenco, and N. P. P. Macciotta. 2020. Variance components using genomic information for 2 functional traits in Italian Simmental cattle: Calving interval and lactation persistency. J. Dairy Sci. 103:5227–5233. https://doi.org/10.3168/jds.2019-17421.
- Costa, A., R. Negrini, M. De Marchi, G. Campanile, and G. Neglia. 2020. Phenotypic characterization of milk yield and quality traits in a large population of water buffaloes. Animals (Basel) 10:327. https://doi.org/10.3390/ani10020327.

- de Araujo Neto, F. R., L. Takada, D. J. A. dos Santos, R. R. Aspilcueta-Borquis, D. F. Cardoso, A. V. do Nascimento, K. M. Leão, H. N. de Oliveira, and H. Tonhati. 2020. Identification of genomic regions related to age at first calving and first calving interval in water buffalo using single-step GBLUP. Reprod. Domest. Anim. 55:1565-1572. https://doi.org/10.1111/rda.13811.
 de Camargo, G. M. F., R. R. Aspilcueta-Borquis, M. R. S. Fortes,
 - R. Porto-Neto, D. F. Cardoso, D. J. A. Santos, S. A. Lehnert, A. Reverter, S. S. Moore, and H. Tonhati. 2015. Prospecting major genes in dairy buffaloes. BMC Genomics 16:872. https://doi.org/ 10.1186/s12864-015-1986-2.
 - de Oliveira Seno, L., H. Tonhati, V. L. Cardoso, E. Faro, R. C. Sesana, and R. R. A. Borquis. 2007. Genetic parameters for milk yield, age at first calving and interval between first and second calving in milk buffaloes. Ital. J. Anim. Sci. 6(Suppl. 2):397–400. https://doi .org/10.4081/ijas.2007.s2.397.
 - Dekkers, J. C. M. 1991. Estimation of economic values for dairy cattle breeding goals: Bias due to sub-optimal management policies. Livest. Prod. Sci. 29:131–149. https://doi.org/10.1016/0301 -6226(91)90062-U.
 - D'Occhio, M. J., S. S. Ghuman, G. Neglia, G. della Valle, P. S. Baruselli, L. Zicarelli, J. A. Visintin, M. Sarkar, and G. Campanile. 2020. Exogenous and endogenous factors in seasonality of reproduction in buffalo: A review. Theriogenology 150:186–192. https:// /doi.org/10.1016/j.theriogenology.2020.01.044.
 - El-Bramony, M. M. 2011. Genetic and phenotypic parameters of milk yield and reproductive performance in the first three lactations of Egyptian buffalo. Egypt. J. Anim. Prod. 48:1–10. https://doi.org/ 10.21608/ejap.2011.94365.
 - Fernandes, P. B., K. O. Marques, F. R. de Araujo Neto, D. P. De Oliveira, N. A. Hurtado-Lugo, R. R. Aspilcueta-Borquis, and H. Tonhati. 2016. Genetic-quantitative study of the first-service pregnancy probability of Murrah heifers. Reprod. Domest. Anim. 51:428–434. https://doi.org/10.1111/rda.12697.
 - Gómez, M., D. Rossi, R. Cimmino, G. Zullo, Y. Gombia, D. Altieri, R. Di Palo, and S. Biffani. 2021. Accounting for genetic differences among unknown parents in *Bubalus bubalis*: A case study from the Italian Mediterranean buffalo. Front. Genet. 12:625335. https:// doi.org/10.3389/fgene.2021.625335.
 - Gupta, J. P., G. K. Sachdeva, R. S. Gandhi, and A. K. Chakaravarty. 2015. Developing multiple-trait prediction models using growth and production traits in Murrah buffalo. Buffalo Bull. 34:347–355.
 - Iamartino, D., E. L. Nicolazzi, C. P. Van Tassell, J. M. Reecy, E. R. Fritz-Waters, J. E. Koltes, S. Biffani, T. S. Sonstegard, S. G. Schroeder, P. Ajmone-Marsan, R. Negrini, R. Pasquariello, P. Ramelli, A. Coletta, J. F. Garcia, A. Ali, L. Ramunno, G. Cosenza, D. A. A. de Oliveira, M. G. Drummond, E. Bastianetto, A. Davassi, A. Pirani, F. Brew, and J. L. Williams. 2017. Design and validation of a 90K SNP genotyping assay for the water buffalo (*Bubalus bubalis*). PLoS One 12:e0185220. https://doi.org/10.1371/journal .pone.0185220.
 - ICAR (International Committee for Animal Recording). 2021. Section 17—Guidelines for Buffalo Milk Recording. Pages 325–35. Online Comm. Anim. Rec., Rome, Italy, 2010. Accessed May 29, 2023. https://www.icar.org/Guidelines/17-Buffalo-Milk-Recording.pdf.
 - Kumar, V., A. Chakravarty, C. Patil, J. Valsalan, and A. Mahajan. 2015. Estimate of genetic and non-genetic parameters for age at first calving in Murrah buffalo. Indian J. Anim. Sci. 85:84–85.
 - Liu, Z., J. Jaitner, F. Reinhardt, E. Pasman, S. Rensing, and R. Reents. 2008. Genetic evaluation of fertility traits of dairy cattle using a multiple-trait animal model. J. Dairy Sci. 91:4333–4343. https://doi.org/10.3168/jds.2008-1029.
 - Lucy, M. C. 2019. Symposium review: Selection for fertility in the modern dairy cow—Current status and future direction for genetic selection. J. Dairy Sci. 102:3706–3721. https://doi.org/10.3168/jds .2018-15544.
 - Lukowski, S. W., L. R. Lloyd-Jones, A. Holloway, H. Kirsten, G. Hemani, J. Yang, K. Small, J. Zhao, A. Metspalu, E. T. Dermitzakis, G. Gibson, T. D. Spector, J. Thiery, M. Scholz, G. W. Montgomery, T. Esko, P. M. Visscher, and J. E. Powell. 2017. Genetic correlations reveal the shared genetic architecture of transcription

in human peripheral blood. Nat. Commun. 8:483. https://doi.org/ 10.1038/s41467-017-00473-z.

- Ma, L., J. B. Cole, Y. Da, and P. M. VanRaden. 2019. Symposium review: Genetics, genome-wide association study, and genetic improvement of dairy fertility traits. J. Dairy Sci. 102:3735–3743. https://doi.org/10.3168/jds.2018-15269.
- Malhado, C., A. Ramos, P. Carneiro, D. Azevedo, P. Affonso, D. Pereira, and J. Souza. 2009. Estimativas de parâmetros genéticos para características reprodutivas e produtivas de búfalas mestiças no Brasil. Rev. Bras. Saúde Prod. Anim. 10:2009.
- Malhado, C. H. M., A. C. M. Malhado, A. A. Ramos, P. L. S. Carneiro, J. C. Souza, and A. Pala. 2013. Genetic parameters for milk yield, lactation length and calving intervals of Murrah buffaloes from Brazil. Rev. Bras. Zootec. 42:565–569. https://doi.org/10 .1590/S1516-35982013000800005.
- Martinez-Castillero, M., H. Toledo-Alvarado, S. Pegolo, A. I. Vazquez, G. de Los Campos, L. Varona, R. Finocchiaro, G. Bittante, and A. Cecchinato. 2020. Genetic parameters for fertility traits assessed in herds divergent in milk energy output in Holstein-Friesian, Brown Swiss, and Simmental cattle. J. Dairy Sci. 103:11545–11558. https: //doi.org/10.3168/jds.2020-18934.
- Mercadante, M. E. Z., R. B. Lôbo, and H. N. Oliveira. 2000. Estimativas de (co) variâncias entre características de reprodução e de crescimento em fêmeas de um rebanho Nelore. Rev. Bras. Zootec. 29:997–1004. https://doi.org/10.1590/S1516-3598200000400008.
- Miglior, F., A. Fleming, F. Malchiodi, L. F. Brito, P. Martin, and C. F. Baes. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. J. Dairy Sci. 100:10251–10271. https://doi.org/10.3168/jds.2017-12968.
- Minervino, A. H. H., M. Zava, D. Vecchio, and A. Borghese. 2020. Bubalus bubalis: A short story. Front. Vet. Sci. 7:570413. https:// doi.org/10.3389/fvets.2020.570413.
- Misztal, I., S. Tsuruta, D. A. L. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, and Z. Vitezica. 2018. Manual for BLUPF90 Family Programs. University of Georgia.
- Misztal, I., S. Tsuruta, T. Strabel, B. Auvray, T. Druet, and D. Lee. 2002. BLUPF90 and related programs (BGF90). Pages 743–744 in Proc. 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France.
- Morammazi, S., R. Torshizi, Y. Rouzbehan, and M. B. Sayyadnejad. 2007. Poster estimates of genetic parameters for production and reproduction traits in Khuzestan buffalos of Iran. Ital. J. Anim. Sci. 6:421–424.
- Muggeo, V. M. R. 2003. Estimating regression models with unknown break-points. Stat. Med. 22:3055–3071. https://doi.org/10.1002/ sim.1545.
- Muggeo, V. M. R. 2008. Segmented: an R package to fit regression models with broken-line relationships. R News 8:20–25.
- Muggeo, V. M. R. 2017. Interval estimation for the breakpoint in segmented regression: A smoothed score-based approach. Aust. N. Z. J. Stat. 59:311–322. https://doi.org/10.1111/anzs.12200.
- Neglia, G., D. de Nicola, L. Esposito, A. Salzano, M. J. D'Occhio, and G. Fatone. 2020. Reproductive management in buffalo by artificial insemination. Theriogenology 150:166–172. https://doi.org/10 .1016/j.theriogenology.2020.01.016.
- Negrini, R., S. Biffani, M. Fioretti, R. Cimmino, and M. Gómez. 2021. Developing a new selection index for the Italian Mediterranean buffalo (*Bubalus bubalis*). Pages 199–203 in Proc. 44th ICAR Annual Conf., Leeuwarden, the Netherlands.

- Pryce, J. E., G. Simm, P. R. Amer, M. P. Coffey, and A. W. Stott. 2000. Returns from genetic improvement in dairy cattle over a twenty-year horizon. Page 37 in Proc. British Society of Animal Science, 2000. Cambridge University Press.
- R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.
- Rathod, A., M. Vaidya, and S. S. Ali. 2018. Genetic studies of productive and reproductive attributes of Surti buffalo in Maharashtra. Int. J. Livest. Res. 8:309. https://doi.org/10.5455/ijlr .20171016061752.
- Robinson, D. L. 2008. Days to calving in artificially inseminated cattle: Alternative models and analyses. Livest. Sci. 117:34–42. https: //doi.org/10.1016/j.livsci.2007.11.010.
- Roxström, A., and E. Strandberg. 2002. Genetic analysis of functional, fertility-, mastitis-, and production-determined length of productive life in Swedish dairy cattle. Livest. Prod. Sci. 74:125–135. https://doi.org/10.1016/S0301-6226(01)00300-1.
- Schaeffer, L. R. 2018. Necessary changes to improve animal models. J. Anim. Breed. Genet. 135:124–131. https://doi.org/10.1111/jbg .12321.
- Shao, B., H. Sun, M. J. Ahmad, N. Ghanem, H. Abdel-Shafy, C. Du, T. Deng, S. Mansoor, Y. Zhou, Y. Yang, S. Zhang, L. Yang, and G. Hua. 2021. Genetic features of reproductive traits in bovine and buffalo: Lessons from bovine to buffalo. Front. Genet. 12:617128. https://doi.org/10.3389/fgene.2021.617128.
- Tonhati, H., F. B. Vasconcellos, and L. G. Albuquerque. 2000. Genetic aspects of productive and reproductive traits in a Murrah buffalo herd in São Paulo, Brazil. J. Anim. Breed. Genet. 117:331–336. https://doi.org/10.1046/j.1439-0388.2000.00249.x.
- VanRaden, P. M., M. E. Tooker, J. R. Wright, C. Sun, and J. L. Hutchison. 2014. Comparison of single-trait to multi-trait national evaluations for yield, health, and fertility. J. Dairy Sci. 97:7952– 7962. https://doi.org/10.3168/jds.2014-8489.
- Visscher, P. M., and M. E. Goddard. 1993. Fixed and random contemporary groups. J. Dairy Sci. 76:1444–1454. https://doi.org/10 .3168/jds.S0022-0302(93)77475-5.
- Weller, J. I. 1989. Genetic analysis of fertility traits in Israeli dairy cattle. J. Dairy Sci. 72:2644–2650. https://doi.org/10.3168/jds .S0022-0302(89)79405-4.
- Weller, J. I., M. Gershoni, and E. Ezra. 2022. Breeding dairy cattle for female fertility and production in the age of genomics. Vet. Sci. 9:434. https://doi.org/10.3390/vetsci9080434.
- Zicarelli, L. 1997. Reproductive seasonality in buffalo. Pages 29–52 in Proc. Third Course on Biotechnology of Reproduction in Buffaloes, Caserta, Italy.

ORCIDS

- M. Gómez-Carpio ⁽⁶⁾ https://orcid.org/0000-0002-3426-2231
- A. Cesarani https://orcid.org/0000-0003-4637-8669
- G. Zullo bhttps://orcid.org/0000-0002-3662-8436
- R. Cimmino Dhttps://orcid.org/0000-0002-0146-1787
- G. Neglia https://orcid.org/0000-0002-0989-6072
- G. Campanile https://orcid.org/0000-0002-3242-7274
- S. Biffani https://orcid.org/0000-0001-5559-3630