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The role of age at first calving in shaping production and reproductive outcomes in Italian buffaloes

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ABSTRACT

The age at first calving (AFC) is a critical parameter in dairy herd management due to its impact on feeding costs, genetic progress, longevity, and thus, farm profitability. With the growing global demand for buffalo milk products, recent advancements in reproductive practices and feeding techniques have contributed to a reduction in AFC in Italy. This study investigates the impact of AFC on the productive and reproductive performance of primiparous Italian Mediterranean buffaloes. Data were collected from 50,661 animals across 115 herds, covering 362,703 first-lactation test-day records from 2013 to 2023. The study analyzed productive traits, including milk yield (kg/d), days to milk peak (DMP, d), ECM (kg/d), fat and protein contents (%), and SCS. Reproductive traits, such as calving interval (CIN, d) and days open (DO, d), were analyzed from 2013 to 2021. Repeated mixed linear models were used to assess the effects of AFC on the aforementioned traits, accounting for DIM, calving year and season, and, when applicable, their interactions. Results highlighted a significant reduction in AFC (from 36 to 35 mo), CIN (from 457 to 447 d), and DO (from 147 to 136 d) over the studied period. Buffaloes with AFC >42 mo produced 0.52 kg/d more milk and 0.90 kg/d more ECM, with slightly higher fat content (+0.04%) compared with those with AFC ≤32 mo. This trend was consistent throughout DIM categories. Somatic cell score was significantly lower in buffaloes with AFC between 32 and 35 mo (2.78), whereas higher SCS was observed in those with AFC ≤32 mo (2.81) or >35 mo (2.80). Buffaloes with AFC comprised between 34 and 37 mo had significantly lower CIN and DO (449–450 and 137–139 d, respectively) compared with those with both lower or greater AFC (454 and 143 d, respectively), whereas DMP was not significantly affected by AFC. As expected, likely due to the out-of-breeding technique, the highest values for both CIN and DO were observed in buffaloes calving the first time in winter and spring (on average 455 and 145 d, respectively), and lower values were recorded for those calving in autumn and summer (on average 449 and 138 d, respectively). Buffaloes with AFC ≤30 mo that calved during winter exhibited significantly greater DO (>150 d) compared with those calving with AFC >42 mo during the same period (140 d). This is likely due to the lower capacity to restore ovarian activity that may reduce fertility in younger buffaloes compared with older ones. Conversely, buffaloes with AFC >42 mo that calved in summer tended to have extended DO (139 d) in respect to those with lower AFC (≤30 mo) in the same period (135 d). This may be due to the interaction between the increased photoperiod and the reduced feed intake caused by summer heat stress, which may have an effect on older buffaloes' fertility. Therefore, AFC between 34 and 37 mo represents the optimal window for Italian Mediterranean buffaloes, combining shorter CIN and DO with sustained milk production. The reduction of AFC could significantly enhance farm profitability and may be achievable through the integration of breeding programs, better reproductive technologies across seasons, and targeted nutritional strategies during the preweaning and prepartum phases.
Key words: days open, fertility, heifers, reproduction management

INTRODUCTION

Buffalo milk production has increased from 67 to 144 million tonnes over the last 2 decades, emerging as an important and growing market within the global dairy industry (FAO, 2024). Italy plays a key role in buffalo milk

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production in Europe, hosting ~3% of the world's buffalo population (FAO, 2024). Around 131,000 lactating buffaloes are monitored annually in Italy (Italian National Association of Buffalo Breeders, 2024), where most of the population is reared in the southern regions to produce mozzarella di Bufala Campana PDO (CLAL, 2023). From 2012 to 2021, mozzarella production increased by 40% (CLAL, 2023). This growth was driven by the rising market value of mozzarella as well as improved farming practices, such as optimized nutrition, reproductive strategies, and enhanced animal welfare, which in turn contributed to greater production efficiency (Neglia et al., 2023).

Among the key factors influencing buffalo and dairy production, heifer management is crucial to ensure long-term productivity at herd level, optimize resource use, and improve overall farm efficiency and profitability (Ferrari et al., 2024; Gómez-Carpio et al., 2025). Heifer rearing, accounting for 15% to 20% of total dairy farm costs (Boulton et al., 2015), constitutes a long-term investment, as animals generate returns only after first calving and the onset of lactation. In Italian buffaloes' population, fertility peaks in autumn and winter due to the reduced photoperiod (D'Occhio et al., 2020), whereas milk demand is higher in spring and summer due to increased need for mozzarella cheese production (Otava et al., 2021). Consequently, this mismatch has led to increased interest in reproductive management in buffaloes to improve fertility in late winter and spring (Baruselli et al., 2003; D'Occhio et al., 2020), leading to the development of the out-of-breeding season mating technique. This technique consists in the interruption of reproduction in some periods of the year to avoid milk production when is not desired (Costa et al., 2020b). However, this can lead to extended calving interval (**CIN**) and age at first calving (**AFC**), highlighting the need to optimize reproductive strategies. Reducing AFC can help lower feeding costs, shorten the CIN, accelerate genetic progress, and improve the overall reproductive efficiency of the herd (Zicarelli et al., 2007; El-Awady et al., 2021; Gómez-Carpio et al., 2023). Moreover, as in dairy cows, managing AFC can influence the retention of productive livestock in the herd, thereby affecting animal productive career (Strapáková et al., 2013; Boulton et al., 2017). Gómez-Carpio et al. (2025) reported that delaying the AFC significantly increased the risk of culling in Italian Mediterranean buffalo population. The same study also reported that a reduction in AFC could be associated with insufficient BCS, which, in turn, might negatively affect postpartum reproductive and productive performance due to negative energy balance. Inadequate dietary energy intake, leading to either excessive or insufficient BCS, is associated with an increased number of inseminations per conception, prolonged calving to conception intervals, and extended calving intervals in younger heifers, ultimately impairing ovarian function and oocyte quality (Campanile et al., 2006; Hussein Ali and Abdel-Raheem, 2013).

Over the past 30 yr, breeding programs and husbandry improvements in Italian Mediterranean buffaloes had successfully reduced the average AFC from 35 to 36 mo (Gómez-Carpio et al., 2025). It is generally well known that the assessment of specific criteria, such as AFC, is a common approach to evaluate the efficiency and profitability of dairy farms (Lopes et al., 2009). However, lower AFC is also associated with potential trade-offs, including reduced milk production during the first lactation, a factor that also contributes to higher culling rates (Rostellato et al., 2021). Although interest in AFC as a management tool in dairy herds is increasing, its longitudinal effect on fertility, milk production, and quality traits during the first lactation in buffaloes, particularly under commercial farming conditions, remains unexplored. Existing studies on buffaloes are often constrained by limited observation periods, further reducing the generalizability of their findings. Moreover, the interaction between AFC and seasonal reproductive dynamics is still poorly understood, hindering the development of targeted reproductive strategies throughout the year. Thus, the present study, based on 10 yr of data collection, aims to investigate the relationship between AFC and both milk production and reproductive performance in a large cohort of primiparous Italian Mediterranean buffaloes to identify the optimal AFC window.

MATERIALS AND METHODS

Ethical approval from an animal welfare and use committee was not required for this study, as data were obtained from routine animal recording practices conducted by the Italian National Association of Buffalo Breeders (ANASB).

Data and Editing

Data included information on routine test-day records of Italian Mediterranean buffaloes from January 2013 to December 2023, including individual data on milk yield (kg/d), stage of lactation (DIM, d), birth and calving dates. Test-day records were taken once a month at fixed intervals within the same herd. Fat and protein contents (%) were estimated from milk spectra using buffalo-specific prediction models in the MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark) at the Italian Breeders Association milk laboratory in Benevento, Italy. Whereas SCC (cells/mL) were determined using Fossomatic (Foss Electric A/S, Hillerød, Denmark). Based on this information, ECM (kg/d) was calculated using the buffalo-specific formula from Campanile et al. (2003):

$$ECM = (\{[Fat\ (\text{g/kg}) - 40 + Protein\ (\text{g/kg}) - 31] \times 0.01155\} + 1) \times Milk\ yield\ (\text{kg/d}).$$

Data were checked for outliers using the productive trait limits outlined in the phenotypic characterization by Costa et al. (2020a). Specifically, milk yield, ECM, fat, and protein content were set as missing values if outside the range of mean ± 3 SD and excluded from the analysis, whereas SCC was retained within 1,000 to 999,000 cells/mL and converted into a linear score (SCS) using the following formula:

$$\text{SCS} = 3 + \log_2(\text{SCC}/100{,}000).$$

Only primiparous buffaloes from herds with continuous monitoring throughout the study period were included. Moreover, to increase the robustness of the subsequent analysis, only primiparous animals with at least 3 test-day records in the first lactation and a minimum of 5 monitored buffaloes per herd-test-day were retained, resulting in 362,703 test-day records from 50,661 buffaloes in 115 farms, primarily reared in southern regions of Italy (90%). Descriptive statistics of productive traits after editing are summarized in Table 1.

Other reproductive traits were derived from previously recorded data: AFC (d) was calculated as the difference between the date of first calving and date of birth; CIN (d) was calculated as the difference between the dates of the second and first calving; conception date was estimated assuming a standard gestation length of 310 d for Italian Mediterranean buffaloes; days open (**DO**, d) were calculated as the difference between the estimated conception date and the first calving date. Values of AFC and CIN were excluded based on the following criteria: AFC <23 or >47 mo, and CIN <350 or >800 d as specified by Gómez-Carpio et al. (2023). These thresholds were applied to remove any possible confounder factors, as fertility traits are highly influenced by nongenetic factors such as out-of-breeding season technique (Gómez-Carpio et al., 2023). Values of DO falling outside the mean ± 0.5 SD were treated as missing, as they were calculated based on a standard gestation length (310 d) to estimate conception dates, rather than using actual conception dates, which were not available. Moreover, because data collection ended in 2023, CIN and DO records from animals that calved after 2022 were excluded from the analysis, as these values could be underestimated due to data truncation. Days to milk peak (**DMP**, d) were determined by identifying the test-day with the highest recorded milk yield value during the first lactation, including only buffaloes with at least one test-day record between 30 and 70 DIM. The DMP was calculated as the interval (d), between the first calving date and the determined peak milk yield test-day.

Table 1. Descriptive statistics of milk yield (kg/d), composition traits (%), ECM (kg/d), and SCS based on individual test-day records of primiparous buffaloes from 2013 to 2023 (n = 362,703)

Trait	n	Mean	SD	Median	IQR[1]
Milk (kg/d)	50,630	8.87	3.18	8.70	4.30
Fat (%)	49,114	8.06	1.76	8.05	2.23
Protein (%)	49,151	4.72	0.43	4.70	0.56
ECM[2] (kg/d)	48,812	14.4	4.69	14.3	6.17
SCS	48,371	2.81	1.66	2.58	2.14

[1]IQR = interquartile range.

[2]ECM calculated using the following formula adapted for buffaloes (Campanile et al., 2003): ({[Fat (g/kg) − 40 + Protein (g/kg) − 31] × 0.01155} +1) × Milk yield (kg/d).

Statistical Analyses

Descriptive statistics for reproductive traits were calculated. Days in milk were categorized into 10 classes of 30 DIM each, except for the first and last categories, which encompassed 5 to 30 DIM and >271 DIM, respectively. Each category accounted for ~12% of the observations. For the same purpose AFC has been converted into categories. The AFC was categorized into 8 categories: ≤30 mo, >30 to ≤32 mo, >32 to ≤34 mo, >34 to ≤35 mo, >35 to ≤37 mo, >37 to ≤38 mo, >38 to ≤42 mo, and >42 mo. These categories were designed to group animals into intervals that reflect both a uniform number of observations and meaningful biological thresholds for reproductive management. The effect of AFC on productive traits was investigated through a mixed linear model with repeated measurements implemented through GLIMMIX procedure in SAS 9.4 software (SAS Institute Inc., Cary, NC):

$$y_{ijklmn} = \mu + AFC_i + DIM_j + Season_k + Year_l + (AFC \times DIM)_{ij} + (AFC \times Season)_{ik} + (DIM \times Season)_{jk} + Animal\_ID_m + HTD_n + e_{ijklmn},$$

where μ is the population mean for the evaluated traits milk yield, fat and protein content, ECM and SCS; AFC_i is the fixed effect of the ith category of buffalo AFC (i = from 1 to 8 categories); DIM_j is the fixed effect of the jth category of DIM (j = from 1 to 10 categories); $Season_k$ is the fixed effect of the kth calving season (k = autumn: September, October, November; winter: December, January, February; spring: March, April, May; summer: June, July, August); $Year_l$ is the fixed effect of the lth calving year (l = from 2013 to 2023); $(AFC \times DIM)_{ij}$ is the fixed interaction effect between AFC and DIM categories; $(AFC \times Season)_{ik}$ is the fixed interaction effect between

AFC categories and calving season; $(DIM \times Season)_{jk}$ is the fixed interaction effect between DIM categories and calving season; $Animal\_ID_m$ is the random effect of the mth Animal ID $\sim N\left(0, \sigma_a^2\right)$, where σ_a^2 is the animal variance; HTD_n is the random effect of the nth herd-test-day $\sim N\left(0, \sigma_a^2\right)$, where σ_a^2 is the herd-test-day variance; and e_{ijklmn} is the random residual $\sim N\left(0, \sigma_e^2\right)$, where σ_e^2 is the error variance. Residual normality and homoscedasticity were assessed using diagnostic plots generated with the PROC GLIMMIX procedure (Supplemental Figures S5, S6, S7, S8, S9; see Notes). Multicollinearity among fixed effects was evaluated before model fitting, using the PROC GLMSELECT. All predictors showed variance inflation factor values below 2, indicating the substantial absence of collinearity.

A similar model was applied to reproductive traits, such as CIN, DO, and DMP excluding the effect DIM categories and their interactions. In this case, only the random effect of herd was included, as these variables were not measured repeatedly within each individual animal, but rather within individuals of the same herds. Additionally, because of the data skewness, a generalized linear mixed model with a gamma distribution and log-link function was used for the analysis of CIN and DO (Diana et al., 2021), implemented through the GLIMMIX procedure in SAS:

$$y_{ijkl} = \mu + AFC_i + Season_j + Year_k + (AFC \times Season)_{ij} + Herd_l + e_{ijkl},$$

where, μ is the population mean for the evaluated traits: CIN, DO; AFC_i is the fixed effect of the ith category of buffalo AFC (i = from 1 to 8 categories); $Season_j$ is the fixed effect of the jth calving season (j = autumn: September, October, November; winter: December, January, February; spring: March, April, May; summer: June, July, August); $Year_k$ is the fixed effect of the kth calving year (k = from 2013 to 2021); $(AFC \times Season)_{ij}$ is the fixed interaction effect between AFC categories and calving season; $Herd_l$ is the random effect of the lth Herd $\sim N\left(0, \sigma_a^2\right)$, where σ_a^2 is the herd variance; and e_{ijklmn} is the random residual $\sim N\left(0, \sigma_e^2\right)$, where σ_e^2 is the error variance. In addition, DMP was analyzed using the same model incorporating the test-day records effect to adjust for the randomness of the sampling date. The goodness of fit of each model was evaluated lowering Akaike's information criterion and bayesian information criterion. Results were presented as least squares means and associated pooled standard error (SEM), and Bonferroni post hoc test ($P < 0.05$) has been conducted for multiple comparisons.

RESULTS AND DISCUSSION

Descriptive Statistics of Productive Traits

Descriptive statistics and phenotypic annual trend of reproductive traits based on least squares means are summarized in Supplemental Figures S1 and S2 (see Notes). Calving interval and DO denote moderate variability, averaging 452 ± 72 and 141 ± 72 d, respectively. Literature reported that reproductive traits can be affected by herd management (Abdalla, 2003; Gómez-Carpio et al., 2023) in relation to the out-of-breeding season mating technique. Heifers that reach puberty in autumn or winter may experience a delayed age at first conception due to the increased demand for buffalo milk at the onset of spring (Campanile et al., 2009), which may lead farmers to postpone breeding to the following spring to align calving with market needs and increase their variability. The results showed a significant decrease in both CIN and DO between 2013 and 2021, with CIN declining from 461 to 447 d and DO from 150 to 136 d (Supplemental Figure S1). These improvements can be attributed to the advancements in feeding strategies and welfare conditions implemented over time in Italian Mediterranean buffalo farms (Campanile et al., 2001). Moreover, the adoption of artificial insemination, as well as advancements in synchronization protocols (Presicce et al., 2022), have contributed to improved reproduction efficiency (Baruselli et al., 2018). Otava et al. (2021), in a study conducted on a single farm between 2015 and 2017, reported an average DO of 113 d for primiparous buffaloes managed with an estrus synchronization protocol. Although their study applied artificial insemination continuously throughout the year, buffaloes in the present study may be influenced by the out-of-breeding season mating technique adopted on-farm. However, a gradual reduction in DO was achieved in our population, making our results comparable to those of Otava et al. (2021).

The average AFC was 35.5 mo, showing a significant reduction from 36 mo in 2013 to 35 mo in 2023 (Supplemental Figure S2), in line with the findings of Gómez-Carpio et al. (2023) in the same population (35 mo). Whereas DMP were stable and did not significantly vary across the years ($P > 0.05$; Supplemental Figure S2). The age of puberty in buffaloes, and thus AFC, may vary according to breed and latitude. For instance, the average AFC reported for Murrah breed buffaloes in India can range between 37 and 54 mo (Rautela et al., 2024). In Italian Mediterranean buffalo, puberty is reported to occur between 17 and 19 mo, with an average AFC of 35 mo at a BW ranging from 340 to 360 kg (Campanile et al., 2009; Gómez-Carpio et al., 2023). Advancements in herd management have led to a gradual reduction in

AFC, decreasing by ~1 mo every 5 yr in Italy, as reported by Zicarelli (2016). Balanced and optimized nutritional management of heifers represents a key factor in this reduction, as it has been demonstrated to be crucial in reproductive management of both cows (Heinrichs et al., 2005) and buffaloes (Campanile et al., 2001). For example, in the study of Campanile et al. (2010) on Italian Mediterranean buffaloes, heifers fed a high-energy diet exhibited higher BCS compared with those receiving a low-energy diet (4.20 vs. 3.40, respectively). The same study reported that the oocytes produced by heifers fed with the low-energy diet were of lower quality. In line with these findings, Baruselli et al. (2001) reported that poor BCS is associated with higher incidence of anestrus in buffaloes, whereas Kumar et al. (2020) observed that low BCS was linked with reduced pregnancy rates. As observed in dairy cows, primiparous buffaloes with low BCS, and consequently lower BW at calving, combined with a relatively high calf weight, may face an increased inflammatory response and slower postpartum recovery (Mammi et al., 2021). Medium BCS has been linked to greater stayability beyond the third lactation in dairy cows (Buonaiuto et al., 2023); however, excessive BCS can lead to calving difficulties. These findings suggest that applying appropriate nutritional strategies to ensure an adequate BCS during heifer rearing could positively affect the future reproductive performance and longevity of buffaloes (Barile, 2005; Sarwar et al., 2009).

Effect of AFC on Productive Performance

F-values and significance (P-value) of fixed effects and interactions influencing milk yield, ECM, fat and protein contents, and SCS are presented in Supplemental Table S1 (see Notes). All the fixed effects significantly explained the variability of the analyzed traits ($P < 0.05$), except for AFC on protein content ($P = 0.05$). The interaction between AFC categories and calving season did not have an effect on protein content or SCS ($P = 0.13$ and $P = 0.66$, respectively; Supplemental Table S1) and DMP ($P = 0.62$; Supplemental Table S2, see Notes). Table 2 provides the least squares means of productive traits for the effect of AFC categories. An increase in AFC was associated with significant increases in milk yield, ECM, and fat content ($P < 0.05$). Specifically, buffaloes with AFC >42 mo produced +0.52 kg/d more milk in their first lactation compared with those with AFC ≤30 mo. Zicarelli et al. (2007), in a study of 953 buffaloes, reported a positive correlation between AFC and milk production during the first lactation, although this effect diminishes in subsequent lactations. Whereas, in a study on 314 Murrah buffaloes, Rautela et al. (2024) found that primiparous animals with an AFC between 60 and 65 mo produced more milk (+182 kg/lactation) and had higher milk peak yields (+2.10 kg/d) compared with those calving between 30 and 35 mo. Similar to milk yield, ECM was 0.90 kg/d higher in buffaloes calving at an AFC >42 mo compared with those with an AFC ≤30 mo. This increase was likely driven by the slightly greater fat content (+0.04%) observed in the >42 mo AFC category. The observed increase in milk production may be attributed to the enhanced physiological maturity, including more advanced mammary gland development, typically associated with primiparous buffaloes calving at greater AFC (Macias and Hinck, 2012; Challana et al., 2014). Primiparous animals with lower AFC may experience reduced development of their mammary secretory tissue, limiting milk production in their first lactation (Sejrsen et al., 2000). Catillo et al. (2002) reported no effect of AFC on either protein or fat contents in a population of 534 Italian Mediterranean buffaloes. In contrast, Ettema and Santos (2004), in a study involving 1,933 Holstein heifers, observed lower protein content in animals with lower AFC. These discrepancies may depend on the population studied and management factors affecting heifers' post-calving period (Atashi et al., 2021). Regarding SCS, the lowest values were observed in buffaloes with an AFC between 32 and 35 mo, whereas higher SCS levels were recorded in animals that calved either earlier or later. On average, the SCS values observed in this study are consistent with those reported by Costa et al. (2020b) for Italian Mediterranean buffaloes, who documented a range between 2.71 to 3.11 for SCS. Previous studies in dairy cattle supported the association between AFC and udder health. Eastham et al. (2018) observed that higher AFC in dairy cows was associated with increased SCC, likely due to prolonged exposure to mastitis pathogens during the precalving period in older heifers, as also suggested by De Vliegher et al. (2012). Similarly, Atashi et al. (2021) reported higher SCS at both 100 and 305 d in Holstein heifers with greater AFC. According to their findings, healthier heifers, which tend to calve earlier, may exhibit greater resilience to mastitis. Mastitis can compromise udder health during the first lactation and this in turn can be reflected throughout their productive life. Indeed, Costa et al. (2019) found that buffalo heifers with high SCS during the first lactation tended to maintain elevated SCS and lower milk yields in subsequent lactations. Furthermore, Eastham et al. (2018) highlighted that higher AFC in dairy cows was associated with reduced average daily lifetime milk production and a lower likelihood of consecutive calvings. Therefore, although higher AFC in buffaloes may be associated with increased milk and fat yields during the first lactation, it could have detrimental effects on udder health and future lactations, suggesting that AFC may be anticipated within 32 to 35 mo.

Table 2. Least squares means and associated pooled SEM for milk yield (kg/d), days to milk peak (d), ECM (kg/d), composition traits (%), and SCS according to the effects of age at first calving (AFC) based on individual test-day records of primiparous buffaloes from 2013 to 2023 (n = 362,703)

	AFC[1] (mo)								
Trait	≤30	30–32	32–34	34–35	35–37	37–38	38–42	>42	SEM
Milk (kg/d)	7.97[e]	8.17[d]	8.27[c]	8.30[bc]	8.35[b]	8.45[a]	8.46[a]	8.49[a]	0.03
Days to milk peak[2] (d)	58	58	58	58	58	58	58	58	0.20
ECM[3] (kg/d)	13.1[e]	13.4[d]	13.6[c]	13.7[bc]	13.8[b]	13.9[a]	14.0[a]	14.0[a]	0.04
Fat (%)	8.18[b]	8.18[b]	8.19[ab]	8.19[ab]	8.19[ab]	8.20[ab]	8.22[a]	8.22[a]	0.01
Protein (%)	4.76	4.76	4.76	4.76	4.76	4.77	4.77	4.76	0.004
SCS	2.81[abc]	2.79[bc]	2.78[c]	2.78[c]	2.81[ab]	2.83[a]	2.80[ab]	2.80[ab]	0.01

[a–e]Least squares means with different superscript letters within a row differ significantly according to Bonferroni post hoc multiple comparison adjustment ($P < 0.05$).

[1]AFC (mo) was categorized into 8 categories: first: ≤30 mo; second: 30–32 mo; third: 32–34 mo; fourth: 34–35 mo; fifth: 35–37 mo; sixth: 37–38 mo; seventh: 38–42 mo; and eighth: >42 mo.

[2]Days to milk peak = number of days from calving to the highest milk yield during the first lactation, calculated as the difference between the date of milk peak and the date of first calving, based on individual test-day records.

[3]ECM was calculated using the following formula adapted for buffaloes (Campanile et al., 2003): ({[Fat (g/kg) − 40 + Protein (g/kg) − 31] × 0.01155} +1) × Milk yield (kg/d).

Effect of Season on Productive Performance

As previously indicated, calving season is a crucial factor in Italian Mediterranean buffalo's husbandry management. Table 3 presents the least squares means of productive traits according to calving season. Buffaloes that calved in winter and spring exhibited significantly higher milk production and ECM compared with those that calved in summer and autumn, consistent with previous findings ($P < 0.05$; Pawar et al., 2012; Zicarelli, 2017). Similarly, de Carvalho et al. (2016) reported that buffaloes calving in winter produced significantly higher total milk yield in respect to summer (8.47 vs. 7.66 kg/d). Consistently, Gómez-Carpio et al. (2023) observed that the calving season effect was particularly evident during the first phase of lactation, with the lowest milk yield recorded in buffaloes that calved in summer. Seasonal variations in DMP were observed as well, with buffaloes calving in spring reaching milk peak earlier (57 d) compared with the other seasons ($P < 0.05$). Notably, DMP significantly increased during summer (59 d), possibly due to the associated heat stress. This seasonal effect, particularly pronounced in southern regions of Italy, may be more evident in primiparous buffaloes, as heat stress can impair feed intake and in turn their energy balance, delaying DMP (Zicarelli, 2017). Furthermore, fat and protein contents in primiparous buffaloes were significantly influenced by calving season, with the highest values observed in autumn ($P < 0.05$; respectively, 8.23% and 4.77%). Because primiparous buffaloes that calved in autumn had the lowest significant milk yield, the increase in fat and protein content may partly result from a reduction in milk volume and a corresponding increase in concentration due to the possible dilution effect (Boro et al., 2018). Moreover, summer heat stress can adversely affect milk production by reducing feed intake, which in turn diminishes milk yield as well as fat and protein contents (Catillo et al., 2002). In addition, SCS was significantly higher in summer and autumn, aligning with findings in the literature (Costa et al., 2021). This pattern parallels that observed in dairy cows, where Wu et al. (2021) documented higher SCS during summer. Supplemental Figure S3 (see Notes) showed the interaction between AFC categories and calving seasons, highlighting that buffalo with higher AFC had greater milk yield, ECM, and fat content across all seasons ($P < 0.05$). Among the possible combinations, buffaloes that calved between 34 and 37 mo achieved a favorable balance of milk production and fat content across seasons. Whereas DMP, protein, and SCS, were not significantly affected by the interaction between calving season and AFC categories (Figure 1 and Supplemental Figure S3, respectively). These findings confirm that an AFC between 34 and 37 mo is preferable to optimize production traits across seasons.

Effect of DIM on Productive Performance

The effects of DIM on productive traits are presented in Table 4. Consistent with previous studies on Italian Mediterranean (Costa et al., 2020a,b) and Murrah breeds (Cerón-Muñoz et al., 2002), milk production and ECM peaked between 31 and 60 d of lactation and progressively declined thereafter ($P < 0.05$) with an average of 56 d (Supplemental Figure S2). In contrast, fat and protein contents had a significant negative peak in correspondence with milk and ECM positive production peaks ($P < 0.05$) and increased throughout lactation, reaching

Table 3. Least squares means and associated pooled SEM for milk yield (kg/d), days to milk peak (d), ECM (kg/d), composition traits (%), and SCS according to the effect of calving season based on individual test-day records of primiparous buffaloes from 2013 to 2023 (n = 362,703)

	Calving season				
Trait	Autumn	Winter	Spring	Summer	SEM
Milk (kg/d)	8.13^{c}	8.46^{a}	8.43^{a}	8.20^{b}	0.03
Days to milk peak[1] (d)	58.0^{b}	58.0^{b}	57.0^{c}	59.0^{a}	0.14
ECM[2] (kg/d)	13.5^{c}	13.9^{a}	13.9^{b}	13.5^{c}	0.03
Fat (%)	8.23^{a}	8.19^{bc}	8.20^{ab}	8.17^{c}	0.01
Protein (%)	4.77^{a}	4.76^{b}	4.76^{b}	4.76^{b}	0.003
SCS	2.81^{ab}	2.77^{c}	2.79^{bc}	2.82^{a}	0.01

[a–c]Least squares means with different superscript letters within a row differ significantly according to Bonferroni post hoc multiple comparison adjustment ($P < 0.05$).

[1]Days to milk peak = number of days from calving to the highest milk yield during the first lactation, calculated as the difference between the date of milk peak and the date of first calving, based on individual test-day records.

[2]ECM was calculated using the following formula adapted for buffaloes (Campanile et al., 2003): ({[Fat (g/kg) − 40 + Protein (g/kg) − 31] × 0.01155} +1) × Milk yield (kg/d).

their highest values in late lactation. Whereas SCS was significantly lower in early lactation but increased as lactation progressed ($P < 0.05$). This was more evident in buffaloes with lower AFC (Supplemental Figure S4, see Notes), likely due to their lower ability to produce milk and manage subsequent pregnancy at the same time, potentially lowering their immune defenses and increasing the risk of mastitis. It has been observed that different AFC in relation to DIM had an effect on milk yield, ECM, and fat content ($P < 0.05$; Supplemental Figure S4, see Notes). Buffaloes' heifers with greater AFC exhibited greater milk yield, fat content, and ECM at peak compared with those that calved with lower AFC. This trend is likely attributable to the higher DMI potential of older heifers, coupled with their more advanced mammary gland development, that both enhance their milk production and metabolic mobilization. Additionally, older heifers, having completed most of their growth, can allocate more energy toward lactation. Similar patterns have been observed in dairy cows, where animals with lower AFC tend to prioritize energy allocation toward growth rather than milk production, resulting in reduced milk yields (Ettema and Santos, 2004; Atashi et al., 2021). Therefore, achieving an appropriate BW at calving may help younger buffaloes sustain milk production, particularly during early lactation. In dairy cattle, BW at calving is strongly influenced by preweaning management; for example, higher milk intake during preweaning period accelerates weight gain, whereas sufficient starter feed intake supports consistent postweaning growth (Diaz et al., 2001; Bach, 2012). Hu et al. (2020) reported that excessive reliance on milk replacers during the weaning transition can negate these growth benefits, leading to reduced BW gain and impaired structural development due to lower nutrient digestibility. In the case of Italian Mediterranean buffaloes, Vecchio et al. (2013) found that a single daily administration of milk replacer during weaning did not adversely affect calf growth or health. Nevertheless, discrepancies in the literature (Omidi-Mirzaei et al., 2015) suggest that outcomes are highly dependent on the quantity and quality of milk replacers used in individual trials, underlining the need for further research to determine optimal feeding strategies for buffalo calves weaning phase. However, preweaning management is often neglected in Italian Mediterranean buffalo farms, primarily due to the high cost of milk replacers, and the labor-intensive requirements of calf rearing.

Replacement buffalo calves are frequently exposed to cold winter temperatures and overcrowded housing conditions, a consequence of seasonal calving concentration, which can compromise their welfare and stayability in the herd (Vecchio et al., 2007a). Moreover, effective precalving management strategies are crucial to optimize AFC in buffaloes (Sarwar et al., 2009). Improving these conditions fosters the development of healthier heifers, better equipped to meet the metabolic demands associated with their first lactation. Indeed, Vecchio et al. (2007a) reported that increasing the level of concentrate supplementation in heifer diets enhanced both fertility and milk production in primiparous buffaloes. Nutritional imbalances during early lactation, such as those leading to SARA in dairy cows, can trigger systemic inflammation and reduce productivity (Plaizier et al., 2012). Although specific evidence in buffaloes remains limited, it is reasonable to assume that comparable mechanisms may underline postpartum challenges in this species, underscoring the need for balanced nutritional management and targeted interventions during early lactation to safeguard metabolic health and overall animal welfare. In this context, as suggested by Cavallini et al. (2022)

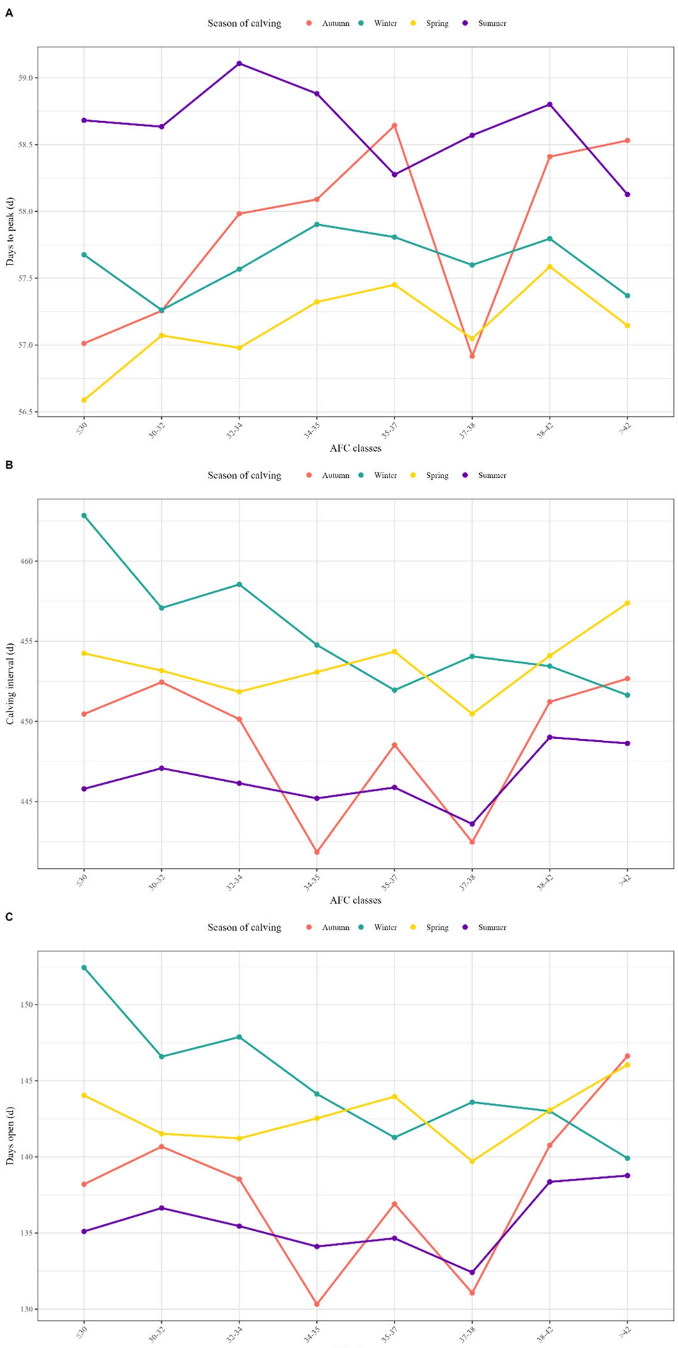

Figure 1. Least squares means of days to milk peak (d), calving interval (d), and days open (d) for the interaction effect of age at first calving (AFC) and calving season. Associated SEM were within the following ranges: 0.11 to 0.23 (days to milk peak), 1.70 to 2.40 (calving interval), and 1.72 to 2.44 (days open). Days to milk peak = number of days from calving to the highest milk yield during the first lactation, calculated as the difference between the date of milk peak and the date of first calving, based on individual test-day records. AFC (months) was categorized into 8 categories: first: ≤30 mo; second: 30 to 32 mo; third: 32 to 34 mo; fourth: 34 to 35 mo; fifth: 35 to 37 mo; sixth: 37 to 38 mo; seventh: 38 to 42 mo; and eighth: >42 mo.

for dairy cows, immunomodulatory feed additives, such as yeast cell wall derivatives, may help mitigate stress by supporting immune function, red blood cell regeneration, and energy balance. Additionally, innovative approaches such as mid-infrared spectroscopy applied to milk analysis have emerged as effective, noninvasive tools for herd-level screening of cows at risk of negative energy balance and subclinical ketosis, thereby supporting more informed decision-making and management strategies at farm level (Magro et al., 2024).

Effect of AFC and Calving Season on Reproductive Performance

Both AFC and calving season had a significant effect on CIN and DO (Supplemental Table S2; $P = 0.03$ and $P = 0.01$, respectively). Although the interaction between AFC categories and calving season did not significantly influence CIN ($P = 0.07$), it had a significant effect on DO ($P = 0.01$). Optimizing AFC is crucial for effective herd management, as studies in cattle have demonstrated that higher AFC is associated with shorter productive lifespan and reduced fertility, ultimately compromising animals' stayability in the herd (Vukasinovic et al., 2001; Strapáková et al., 2013). In the present study, primiparous buffaloes that calved with AFC between 34 and 37 mo had the shortest CIN and DO (on average 449 and 138 d, respectively; Table 5), whereas both higher (>37 mo) and lower (≤34 mo) AFC groups recorded extended CIN and DO. Rautela et al. (2024), in their study on Murrah breed, have reported significantly shorter CIN in buffaloes that calved with AFC between 36 and 47 mo (~470 d). Although calving to first service, services per conception, and calving to conception interval were not affected, these parameters exhibited numerically lower values within the same AFC category in the same study. These differences could be due to the generally greater AFC observed in Murrah breed when compared with Italian Mediterranean buffaloes. Additionally, variations in feeding strategies and management practices between production systems may further explain those increased AFC values. Nevertheless, in both production systems, enhancing reproductive performance can significantly improve farm profitability, primarily by enabling an earlier onset of milk production and consequently shortening the nonproductive rearing period. In the present study, reducing AFC between 34 and 37 mo lead to better reproductive performance, thereby supporting higher lifetime productivity and enhancing the economic sustainability of buffalo farming. In dairy cows, reducing AFC from 26 to 24 and 22 mo increased the net return by \$41.5 and \$24 per heifer, respectively, whereas delaying AFC beyond 26 mo led to reduced profitability due to increased rearing costs (Pirlo et al., 2000).

Table 4. Least squares means and associated pooled SEM for milk yield (kg/d), ECM (kg/d), composition traits (%), and SCS according to the effects of DIM[1] based on individual test-day records of primiparous buffaloes from 2013 to 2023 (n = 362,703)

	DIM										
Trait	5–30	31–60	61–90	91–120	121–150	151–180	181–210	211–240	241–270	271–305	SEM
Milk (kg/d)	9.55[d]	10.8[a]	10.5[b]	9.65[c]	8.84[e]	8.10[f]	7.42[g]	6.71[h]	6.06[i]	5.50[j]	0.03
ECM[2] (kg/d)	14.6[d]	16.2[a]	16.1[b]	15.4[c]	14.5[e]	13.8[f]	13.0[g]	12.0[h]	11.1[i]	10.3[j]	0.03
Fat (%)	7.04[j]	7.09[i]	7.4[h]	7.78[g]	8.11[f]	8.47[e]	8.76[d]	8.97[c]	9.11[b]	9.25[a]	0.01
Protein (%)	4.74[f]	4.50[i]	4.50[i]	4.58[h]	4.67[g]	4.77[e]	4.86[d]	4.94[c]	5.00[b]	5.05[a]	0.003
SCS	2.47[i]	2.34[j]	2.52[h]	2.71[g]	2.82[f]	2.88[e]	2.94[d]	3.00[c]	3.10[b]	3.19[a]	0.01

[a–j]Least squares means with different superscript letters within a row differ significantly according to Bonferroni post hoc multiple comparison adjustment ($P < 0.05$).

[1]DIM was categorized into 10 intervals of 30 DIM each, except for the first and last categories, which encompassed 5 to 30 DIM and >271 DIM, respectively.

[2]ECM was calculated using the following formula adapted for buffaloes (Campanile et al., 2003): ({[Fat (g/kg) − 40 + Protein (g/kg) − 31] × 0.01155} +1) × Milk yield (kg/d).

Typically, primiparous buffaloes show longer DO, CIN, and requires more services per conception, with these parameters decreasing in subsequent parities (de Carvalho et al., 2016). However, CIN in buffaloes is also influenced by reproductive management strategies, synchronization protocols, and seasonal variations (Rossi et al., 2014). In this study, both CIN and DO were significantly influenced by calving season (Table 6), with the longest intervals recorded in buffaloes that calved during winter and spring (on average 455 and 145 d, respectively), compared with those calving in autumn and summer (on average 449 and 138 d, respectively; $P < 0.05$). Similarly, Zicarelli et al. (2007) reported seasonal variations in CIN, observing longer intervals for buffaloes calving between late autumn and early spring (478–488 d), and shorter intervals for those calving from late spring to early autumn (460–468 d). These higher values for CIN could be partly explained considering that their study was conducted more than 10 yr ago, and reproductive performance traits have since improved due to advancements in management and breeding strategies (Supplemental Figure S1). Mating during the out-of-breeding season period reduces fertility in buffaloes, as calving is confined to 7 to 8 mo of the year, corresponding to the least favorable reproductive period for the species. To enhance reproductive efficiency, natural mating is recommended during the transition and nonbreeding season, given the reduced success of assisted reproductive techniques during these periods (Vecchio et al., 2007b). Moreover, younger buffaloes (AFC ≤30 mo) that calved in winter tended to exhibit longer CIN and significantly prolonged DO (>150 d) compared with older buffaloes (AFC >42 mo) calving in the same season (140 d; Figure 1). This may be attributed, at least in part, to their lower BW and reduced BW reserves, which can delay the resumption of ovarian activity (Dash et al., 2016), particularly in late winter when increasing daylight begins to negatively influence reproductive physiology. Indeed, photoperiod is a well-documented factor affecting reproduction in buffaloes, especially at higher latitudes (Campanile et al., 2010). Accordingly, buffaloes calving in winter and spring may experience delayed ovarian reactivation and reduced conception rates due to seasonal anestrus, with the duration of anestrus closely linked to increasing day length (Campanile et al., 2009). Moreover, extended photoperiods have also been associated with higher rates of embryonic loss and prolonged anestrus in Italian Mediterranean buffaloes (Vecchio et al., 2007b).

Buffaloes with greater AFC (>42 mo) calving in summer tended to exhibit longer CIN and had significantly ($P < 0.05$) prolonged DO (449 and 139 d, respectively) compared with their younger counterparts (≤30 mo)

Table 5. Least squares means and associated pooled SEM for calving interval (d) and days open (d) according to the effect of age at first calving (AFC)[1] for primiparous Mediterranean Buffaloes monitored from 2013 to 2021

	AFC (mo)								
Trait	≤30	30–32	32–34	34–35	35–37	37–38	38–42	>42	SEM
Calving interval (d)	454[a]	453[a]	453[a]	449[b]	450[b]	451[a]	453[a]	454[a]	2.07
Days open (d)	143[a]	141[a]	141[a]	137[b]	139[b]	139[a]	139[a]	143[a]	2.09

[a,b]Least squares means with different superscript letters within a row differ significantly according to Bonferroni post hoc multiple comparison adjustment ($P < 0.05$).

[1]AFC was categorized into 8 categories: first: ≤30 mo; second: 30–32 mo; third: 32–34 mo; fourth: 34–35 mo; fifth: 35–37 mo; sixth: 37–38 mo; seventh: 38–42 mo; and eighth: >42 mo.

Table 6. Least squares means and associated pooled SEM for calving interval (d) and days open (d) according to the effect of calving season

	Calving season				
Traits	Autumn	Winter	Spring	Summer	SEM
Calving interval (d)	450[b]	456[a]	455[a]	447[b]	1.84
Days open (d)	139[b]	146[a]	144[a]	137[b]	1.84

[a,b]Least squares means with different superscript letters within a row differ significantly according to Bonferroni post hoc multiple comparison adjustment ($P < 0.05$).

calving in the same season (446 and 135 d, respectively; Figure 1). During summer, elevated temperatures can compromise feed intake, metabolic efficiency, and overall energy balance, particularly in older buffaloes (Marai and Haeeb, 2010). Although buffaloes are generally more heat-tolerant than cattle (Dash et al., 2016), prolonged exposure to heat stress, combined with seasonal nutritional challenges such as lower forage quality and availability, can exacerbate negative energy balance and metabolic stress. These conditions can affect reproduction, impairing ovarian activity and delaying the resumption of reproductive cycles, ultimately extending CIN and DO (D'Occhio et al., 2020). This highlights the complex interplay between environmental stressors and physiological status, indicating that seasonal effects on productive and reproductive performance are multifactorial. For instance, Salzano et al. (2019) demonstrated that increasing day length, another seasonal factor, negatively affects ovarian function in buffalo heifers by reducing both the number of follicles and cumulus-oocyte complexes, whereas an improved metabolic status is associated with better oocyte quality and reproductive outcomes. Taken together, these findings emphasize the need for integrated approaches that combine effective management practices with genetic selection to mitigate the seasonal challenges affecting reproductive performance.

In this regard, selective breeding strategies offer promising avenues to reduce AFC, considering the moderate to low heritability estimates reported for this trait in both Murrah buffaloes (0.15–0.48; Tamboli et al., 2022; Dos Santos et al., 2025) and Italian Mediterranean buffaloes (0.07–0.13; Gómez-Carpio et al., 2023). Notably, Gómez-Carpio et al. (2023) identified a favorable positive genetic correlation between AFC and first CIN (0.10 and 0.41), indicating that selection for earlier AFC would have a favorable effect on reduction of CIN. In this context, the adoption of precision livestock farming (**PLF**) technologies represents a valuable complementary approach during season with lower fertility. Tools such as wearable collars with integrated heat detection sensors enable more accurate and timely estrus identification, improving insemination timing and conception rates (Lamanna et al., 2025). Moreover, PLF technologies may enhance phenotype recording accuracy through continuous data collection, thereby accelerating at the same time the progress in breeding programs.

CONCLUSIONS

This study provides new insights into the optimal management of AFC in Italian Mediterranean buffaloes, identifying the range of 34 to 37 mo as the most favorable balance between productive and reproductive performance. Our findings highlight that both early and delayed AFC can compromise reproductive efficiency, underscoring the critical role of heifer nutrition, BCS, and breeding seasonality in achieving reproductive outcomes. Further research should focus on strategies to further reduce AFC through improved feeding regimens and management strategies, while also exploring season-specific reproductive technologies to enhance conception rates. Finally, integrating reproductive traits into breeding programs, alongside traditional milk yield criteria, could accelerate genetic progress and strengthen the resilience of buffalo farming systems.

NOTES

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Nonstandard abbreviations used: AFC = age at first calving; CIN = calving interval; DO = days open; DMP = days to milk peak; PLF = precision livestock farming.

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