

Effect of parity and season of calving on the postpartum reproductive activity of water buffalo cows

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Abstract

The aim of this study was to determine the effect of parity and season of calving on the probability of water buffalo cows to be mounted for first time before 60 and 100 days postpartum (dpp), and on the length of calving-to-first-mount interval. A retrospective analysis of reproductive records of 1,231 water buffaloes with 5,336 postpartum mounts (2,948 first mounts), was carried out. Probability of water buffaloes to be mounted for first time before 60 or 100 dpp was significantly lower in primiparous (0.165 vs 0.3 and 0.32 vs 0.527, respectively) and in those calving between March-August (0.134 vs 0.354 and 0.312 vs 0.536, respectively) in comparison with multiparous and those calving between September-February. Additionally, it was observed that primiparous water buffalo cows had lower odds (2.13 and 2.38 times lower) and those calving between September-February had higher odds (3.55 and 2.56 times higher) to be mounted ≤ 60 and ≤ 100 dpp, respectively, in comparison with multiparous animals and those calving between March-August. Most mounts (70.6%, $P < 0.0001$) were observed during September and February. Days to first mount were shorter in multiparous water buffalo cows (132.9 vs 162.8; $P < 0.0001$) and in those calving between September-February (140.1 vs 155.6; $P < 0.0001$). In conclusion, primiparous water buffaloes and those calving between March-August show a higher risk of a delay in the resumption of postpartum reproductive activity with a low probability to be mounted for the first time ≤ 60 or ≤ 100 dpp, which involves a longer calving-to-first-mount interval.

Keywords: water buffaloes, parity, season, postpartum cyclicality, anestrus.

Introduction

The world population of water buffalo has grown steadily, and there are currently more than 200 million heads. This positive trend has also been seen in America with an increase of 26.4% between 2005 and 2016 (Borghese et al., 2016). The population of water buffaloes on the American continent is more than 4 million heads and Venezuela ranks second after Brazil with 960,000 animals (Crudeli et al., 2016). This positive trend could be motivated by the fact that dairy water buffaloes have a productivity that is comparable to cows in tropical zones, and by the characteristics of their milk, which is very important for the cheese industry given the highest total solids content; and yields a higher price compared to cow's milk. To maximize the profitability of water buffalo systems, a high milk yield with a high price must be accompanied by a good reproductive performance, because the latter has a huge impact on the economic results (Sweers et al., 2014; Cicek et al., 2017), with a calving interval of 12–13 months being recognized as the economic optimum (Shah et al., 1991).

Longer calving intervals are related to lower milk yield by day of calving interval and to economic losses (Shah, 2007; Sweers et al., 2014; Nava-Trujillo et al., 2018). Recently, a loss of 6.07 USD per extra day in the calving interval has been reported (Cicek et al., 2017). To reach one-year calving interval, the calving to conception interval must be no longer than 60 days (Baruselli & Carvalho 2016). However, the optimum calving to conception interval could be a challenge because reproductive activity of water buffalo cows is affected by several factors and the postpartum anestrus could be an important limiting factor (Nanda et al., 2003; Kumar et al., 2013; Himerath & Ramesha, 2015).

Season of calving (Abayawansa et al., 2011; Devkota et al., 2012), parity (Abayawansa et al., 2011), body condition score (Baruselli et al., 2001; Devkota et al., 2012; Patel et al., 2018), loss of body weight and body condition during the postpartum period (Mavi et al., 2011; Abayawansa et al., 2012) and suckling (Singh & Brar, 2008)

have been reported to lengthen the interval to the first postpartum estrus in water buffaloes. In addition, given the increase of milk yield observed in recent years in the water buffalo species (Fooda et al., 2010; Ahmad et al., 2009; Menéndez-Buxadera & Verde 2014), the evaluation of reproductive performance and the identification of risk and protecting factors, is more necessary to prevent reproductive failures. In fact, it has been reported an adverse relationship between milk yield and reproductive performance in water buffaloes (Abayawansa et al., 2011; Nava-Trujillo et al., 2018; Jamuna et al., 2016).

Little research to understand the factors affecting the reproductive performance of water buffalo in Venezuela, especially about the resumption of postpartum reproductive activity, has been undertaken. Therefore, the objective of this study was to determine the effect of parity and season of calving on the probability of buffalo cows to have the first mount during the first sixty and one-hundred days postpartum (dpp), and on the length of calving-to-first-mount interval.

Materials and methods

This was a retrospective study analyzing reproductive records of 1,231 crossbred (Murrah/Mediterranean) water buffalo cows from one herd, including a total of 5,336 postpartum mounts, of which 2,948 were first mounts, during a period of ten years (2005 – 2015). The farm was located in Jesus María Semprún county, Zulia State, Venezuela. Animals grazed in pastures consisting of *Brachiaria humidicola* and *Brachiaria arrecta* in one- to two-day paddock rotations, with access to a commercial mineral block *ad libitum*, as a dietary supplement. Mechanical milking was performed twice a day after a short sucking by the water buffalo's own calf to stimulate milk letdown. During the milking, the calf remained bound to the water buffalo forelimb, and after milking the calves suckled their mothers to remove residual milk from the udder. A water buffalo bull for each 20–25 cows was present 24 hours during the year-round. Estrus

activity (as observed mount) was monitored by trained personnel previous to each milking and during the grazing, twice a day for one hour each time. The farm kept paper records of each water buffalo cow.

Statistical analysis

Information about parity, month of calving, calving order, and day of estrus (as observed mount) was abstracted from the records. Date of calving and date of observed mount were recorded in the format dd/mm/yyyy. From the date of calving the corresponding month was obtained and a categorical variable called season of calving was created with the following values: March-August (Spring-Summer, long photoperiod) and September-February (Autumn-Winter, short photoperiod). The main predictors were parity (categorized as primiparous and multiparous) and season of calving. The outcome of interest was the calving-to-first-mount interval, both as a continuous variable, computed from the difference between date of calving and date of the first observed breeding, and as 2 different binary variables (cut-off values: 60 days and 100 days, respectively).

The log odds of having a calving-to-first mount interval of ≤ 60 dpp were regressed on parity and season using a logistic regression. The logistic regression model was estimated using a GEE approach, an independent working correlation, and robust standard errors (SAS). A similar model was built for the log odds of having a calving-to-first-mount interval of ≤ 100 dpp (SAS). A linear mixed model of the mean calving-to-first mount interval was built with parity and season of calving as fixed effects and as random coefficients (SAS). Robust standard errors were used to account for heteroskedasticity conditional on the random effects. Multiple comparisons from the previous models were adjusted by Sidak's method, in which the significance level to reject the null hypothesis is $1-(1-\alpha)^{1/k}$ (α : familywise error rate, 0.05; k: number of preplanned comparisons) (Vittinghoff et al., 2012). To determine the distribution of mounts per month, a total of 5,336 events were used in an unconditional multinomial logistic regression with robust standard errors

calculated at the cluster level (water buffalo cow) (Stata). Differences were considered statistically significant when P values were ≤ 0.05 . Predicted probabilities from the logistic regression models were calculated applying the expit function to the predicted log odds (SAS) or through the command margins (Stata).

Results

The grand probability (not conditioning on any predictor) of having the first mount ≤ 60 dpp was 0.298 (95% CI: 0.278, 0.318). The grand probability of having the first mount ≤ 100 dpp was 0.478 (95% CI: 0.456, 0.5). Probabilities of having the first mount ≤ 60 dpp or ≤ 100 dpp were lower in primiparous than in multiparous water buffalo cows (Table 1). Correspondingly, the odds of having the first mount ≤ 60 dpp were 53% lower (95% CI: 61% lower, 45% lower; $P < 0.0001$) in primiparous water buffalo cows than the odds in multiparous water buffalo cows. Similarly, the odds of having the first mount ≤ 100 dpp were 58% lower (95% CI: 64% lower, 50% lower; $P < 0.0001$) in primiparous water buffalo cows than the odds in multiparous water buffalo cows. The distribution of mounts varied between the studied seasons, with a higher frequency between September-February with 70.6% (95% IC: 69.2, 71.9, $P < 0.0001$) (Fig. 1). Season of calving affected the probability of having the first mount ≤ 60 dpp or ≤ 100 dpp (Table 1). The odds of having the first mount ≤ 60 dpp in water buffalo cows calving between September and February were 3.6 times (95% CI: 2.8 times, 4.5 times; $P < 0.0001$) the odds in water buffalo cows calving between March and August. Similarly, the odds of having the first mount ≤ 100 dpp in water buffalo cows calving between September and February were 2.6 times (95% CI: 2.1 times, 3.1 times; $P < 0.0001$) the odds in water buffalo cows calving between March and August.

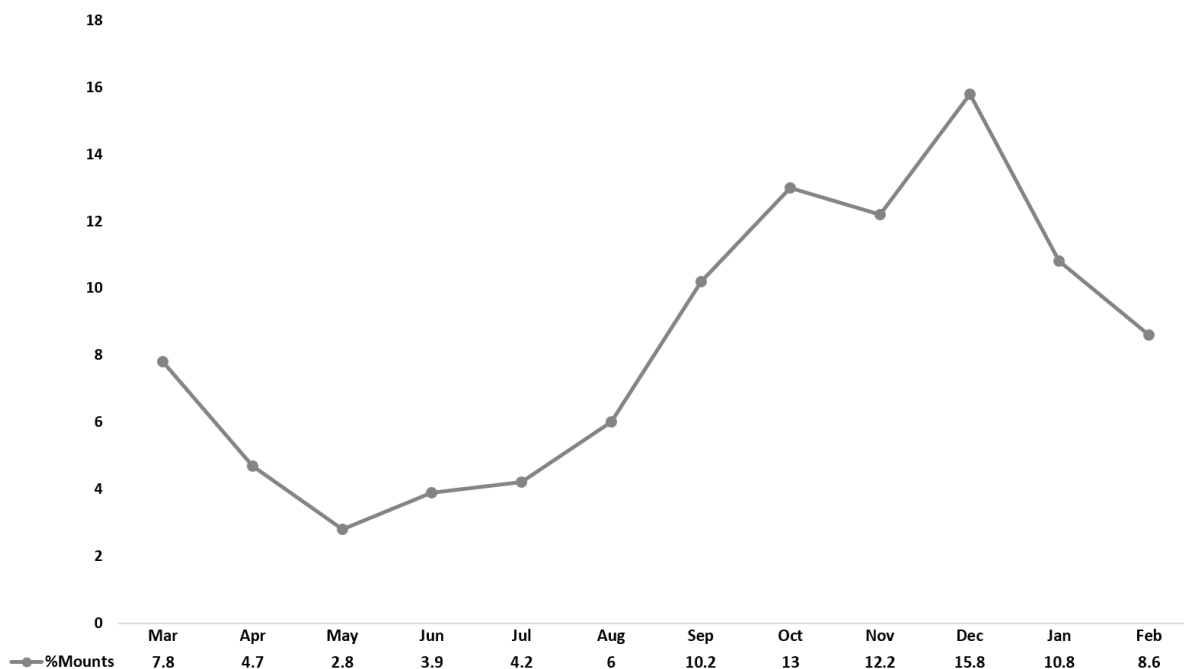
The grand mean of calving-to-first-mount interval was 148.2 days (95% CI: 142.8, 153.6). Parity and season of calving had a significant effect on the calving-to-first-mount interval, with primiparous water buffalo cows having 29.8

Table 1. Effect of parity and season of calving on adjusted probabilities of having the first mount ≤ 60 dpp and ≤ 100 dpp

		≤ 60 dpp		≤ 100 dpp	
		Adjusted probability	95% CI	Adjusted probability	95% CI
Parity	Primiparous	0.165 ^b	0.145, 0.188	0.32 ^b	0.293, 0.348
	Multiparous	0.3 ^a	0.272, 0.33	0.527 ^a	0.497, 0.556
Season	September-February	0.354 ^a	0.33, 0.379	0.536 ^a	0.512, 0.561
	March-August	0.134 ^b	0.112, 0.16	0.312 ^b	0.28, 0.346

95% CI's adjusted for clustering using robust standard errors.

Values in the same column with different lowercase superscripts are significantly different ($P < 0.05$)

**Fig. 1.** Adjusted proportions of mounts per month

extra days (95% CI: 20.3 extra days, 39.4 extra days) than multiparous water buffalo cows. Water buffalo cows calving between September-February had 15.5 days less (95% CI: 25.3 days less, 5.8 days less) than those calving between March-August (Table 2).

Discussion

An early resumption of postpartum reproductive activity is required to achieve a 12- to 13-month calving interval, which is the optimum economic (Shah et al., 1991). Therefore, we

Table 2. Effect of parity and season of calving on adjusted means of calving-to-first-mount interval.

		Adjusted mean	95% CI ¹	Adjusted mean difference ²	95% CI	P value
Parity	Primiparous	162.8	155.2, 170.3	29.8	20.3, 39.4	< 0.0001
	Multiparous	132.9	126.5, 139.4			
Season	March-August	155.6	148.3, 162.9	-15.5	-25.3, -5.8	0.0019
	September-February	140.1	133.2, 146.9			

¹95% CI's were also adjusted for clustering using random coefficients.

²The reference groups were multiparous (for parity) and March-August (for season) and P values and 95% CI's were adjusted for multiple comparisons using Sidak's method.

evaluated how parity and season of calving affected the probability of water buffalo cows to have the first mount ≤ 60 and ≤ 100 dpp, and the length of calving-to-first-mount interval. In addition, the distribution of mounts (the most reliable indicator of estrus; Vale and Riberio, 2005; Suthar & Dhama, 2010) throughout the year was described.

In the present study, postpartum anestrus was confirmed to be a problem with high incidence affecting the reproductive performance of water buffaloes, as it was noticed previously (Kumar et al., 2013). However, present results contrast with Barkawi et al. (1986), who observed that 93% of Egyptian water buffaloes had ovulatory cycles during the first 60 dpp, and with Rojas (2016) and Hassan et al. (2017), who observed a shorter calving-to-first-service or mount intervals. In addition, in the present study a higher probability to be mounted for first time ≤ 100 dpp in comparison to the probability to be mounted for first time ≤ 60 dpp, was observed. Khan et al. (2011) observed that the risk of first service before 60 dpp was lower than the risk of first service before 100 dpp in both winter and summer seasons. Souza et al. (2005) and Shah (2007) observed 53.84% and 84.2%, respectively, of water buffaloes having the first estrus before 100 dpp, while Qureshi & Ahmad (2008) observed that 68.63% of water buffaloes expressed estrus during the first 150 dpp.

Probably our results are related to the postpartum negative energy balance, a low body condition score, and suckling. Although the negative energy balance in postpartum water buffalo has been observed for being shorter in comparison to that of dairy cows, and confined to the first four weeks postpartum (Golla et al., 2019); a low body condition score and an excessive loss of body weight during postpartum period have been related with a longer calving-to-first-estrus and calving-to-conception intervals (Mavi et al., 2011; Abayawansa et al., 2012; Banu et al., 2012; Patel et al., 2018). In addition, the increase in the probability to be mounted in the first 100 dpp observed in this study could be related to the reduction of negative energy balance, the increase in dry matter intake, body condition score, and body weight, and the decrease in milk yield, observed as postpartum progresses (Infascelli et al., 2003; Abayawansa et al., 2012; Deka et al., 2014).

Under the conditions of this study, suckling was used to induce milk letdown and facilitate mechanical milking, which could contribute to the low probability of buffalo cows being mounted during the early postpartum, and to the observed long interval to the first mount. Rijasnaz et al., (2014), observed that 87.5% of buffalo cows whose calves were weaned were cyclic at 90 dpp and had an interval to first estrus of 53.28 ± 7.52 days in comparison with the 0% and 128 ± 9.94

days respectively, to those buffalo cows suckling their calves twice a day, as it was done in the present study. Recently, it was observed that buffalo cows suckling had 37 extra days to first estrus in comparison with those whose calves were weaned (Kantharaja et al., 2018). Although the mechanism through which the suckling affects reproductive performance of water buffalo cows has been scarcely explored, in the case of cows, the bond between the calf and its mother along with factors of ovarian origin, reduces the secretion of luteinizing hormone, which in turn lengthens the anestrus period (Garcia-Winder et al., 1984; Silveira et al., 1993). However, the intensity of this bond decreases as the postpartum period progresses, allowing the cow to resume ovarian activity (Garcia-Winder et al., 1984), and this is in agreement with the increase in probability to be mounted before 100 dpp observed in the present study.

The effect of parity on reproductive performance of water buffaloes has been reported previously. Presicce et al. (2005) observed that primiparous water buffaloes tended to have a lower rate of ovulation in the first 60 dpp in comparison with multiparous, 40% vs 80% ($P = 0.07$) and 10 extra days in the interval calving to first ovulation ($P = 0.07$). Thiruvankadan et al. (2014), Rojas (2016) and Hassan et al. (2017) observed that primiparous water buffaloes had more days to first service, more open days, more services per conception, and in consequence, a longer calving interval. Similar results were observed by Abayawansa et al. (2011), who reported a negative correlation between parity and interval to first postpartum estrus ($r = -0.24$). Probably the low reproductive performance of primiparous buffaloes is a consequence of being still growing and having a stronger postpartum negative energy balance that further delays the resumption of postpartum reproductive activity (Bolivar Vergara et al., 2010; Vecchio et al., 2007). In addition, it has been suggested that anestrus in primiparous buffaloes could be a consequence of the stress of first lactation, as well as bad management in the prepuberal period and in the course of their first pregnancy (Zicarelli, 1997).

Water buffaloes are a short-day species and reproductive activity increases as photoperiod decreases (Zicarelli, 1997; Sampaio Neto et al., 2001; Vale, 2007; Gasparrini, 2019) and this was observed in the present study. In addition, the probabilities of first mount occurring ≤ 60 and ≤ 100 dpp were higher in water buffaloes calving between September-February, which also had a shorter interval to the first mount (Table 2). Therefore, our results corroborate the negative relationship between photoperiod and reproductive activity in water buffalo cows. Reduction of the photoperiod increases the secretion of melatonin (Parmeggiani et al., 1992), which in turn stimulates the secretion of GnRH and gonadotropins promoting ovarian activity (Nowak & Rodway, 1985), while a long photoperiod increases prolactin secretion, resulting in the suppression of the secretion of gonadotropins, which promotes the anestrus (Mondal et al., 2007; Das & Khan, 2010). A higher incidence of anestrus has also been observed during summer in comparison with winter (Rahman et al., 2012; Kumar et al., 2013). In Venezuela, it was observed that water buffaloes calving between July and November had the shortest calving to first service interval while the longest was observed in those calving between February and June (Rojas, 2016). Under Egyptian conditions, water buffaloes calving in autumn and winter had a short interval to first service (71.16 and 63.79 days, respectively, $P < 0.001$) in comparison with those calving in summer and spring (85.57 and 132 days, respectively) (Hassan et al., 2017). Although the photoperiod is a decisive factor to the reproductive seasonal behavior of water buffalo cows, it has been suggested that other factors, such as temperature, humidity, rainfall, and forage supply could affect the reproductive response of water buffalo cows to the season (Sertu et al., 2012; Sánchez et al., 2017). Therefore, more research is warranted, especially because season of calving affects the reproductive performance and in consequence the economic performance of buffalo systems (Hassan et al., 2017), but also because there is the need to break the reproductive seasonality to keep a uniform milk supply throughout the year in some countries, and this practice could reduce

the reproductive performance (Zicarelli, 2017; Gasparini, 2019).

In this study, mounting was used as a signal of resumption of postpartum reproductive activity, but was only detected during the day. Nevertheless, this could be considered suboptimal, because water buffaloes can present a high incidence of estrus during the night (Vale & Ribeiro, 2005) and silent estrus (Qureshi & Ahmad, 2008; Banu et al., 2012; Rahman et al., 2012). Therefore, it is possible that estruses and mounts were not detected or observed as it has been previously suggested (Qureshi & Ahmad, 2008). Banu et al. (2012), observed a low rate of estrus detection (28%) and that the number of missed estruses per buffalo cow was higher between 30 and 70 dpp (2.55) in comparison with those between 71 and 90 dpp (1.33) and between 91 and 130 dpp (0.65). Additionally, it was observed that the interval to first estrus increased as the number of *missed* estruses increased. However, we think that the number of water buffalo cows and observations included in this study are enough to consider our results robust.

Anestrus has been shown to be a problem with high incidence, affecting the reproductive performance of water buffaloes, with primiparous and those water buffaloes calving between March and August, when the photoperiod is longer, being groups with higher risk of this condition. This situation lengthens the calving interval and reduces the profitability (Shah, 2007). Therefore, breeders must take into account these groups and implement practices to reduce the incidence and the duration of postpartum anestrus. Improving estrus detection, increasing the time of observation during the cooler part of the day and night hours (Das & Khan, 2010; Banu et al., 2012; Rao et al., 2013), increasing energy density in the diet and improving body condition scores (Vecchio et al., 2007; Banu et al., 2012; Abdel-Latif et al., 2016), use of additives such as vitamins, minerals, or niacin (Ezzo, 1995; Panda et al., 2006; Khan et al., 2015), reduction of heat stress (Zicarelli, 2017), decreasing the incidence of mastitis, which has been reported to reduce reproductive performance (Manimaran et al., 2014; Mansour et al., 2016), use of hormone

treatments for fixed-time artificial insemination (Hoque et al., 2014; Gutiérrez et al., 2017; Monteiro et al., 2018) and practicing the early weaning (Usmani et al., 1990; Qureshi et al., 2008; Rijasnaz et al., 2014; Kantharaja et al., 2018) are some of the alternatives to reduce the duration of anestrus period and reach the optimum calving interval (12-13 months; Shah et al., 1991).

Conclusions

Postpartum anestrus is an important problem reducing the reproductive performance of water buffaloes, with primiparous and those water buffaloes calving during March-August being groups with higher risk of a delay in the resumption of postpartum reproductive activity with a low probability to be mounted for the first time ≤ 60 or ≤ 100 dpp, which involves a longer calving-to-first-mount interval.

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