Probability of pregnancy before ninety days postpartum in water buffaloes

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Abstract. This study aimed to assess the effect of parity and season of calving on the probability of water buffalo cows becoming pregnant in the first 90 days (90 days) postpartum. A retrospective analysis of 3.181 pregnancies from 1.465 water buffaloes was carried out. Buffaloes were grouped according to parity in one, two, or three and more calvings. Season of calving was established as follows: long photoperiod (March-August) and short photoperiod (September-February). Predicted probabilities from the mixed-effects logistic regression model were calculated, and a generalized linear mixed model was fitted with random intercepts to calculate the log odds of becoming pregnant 90 days postpartum. The probability of pregnancy 90 days postpartum was 0.3645, and it was lower in primiparous (0.2717) than in two-calved (0.3863) and three or more calving (0.5166). Probability of pregnancy 90 days postpartum increased 1.77 odds by each increase in parity. The probability of becoming pregnant 90 days postpartum was greater in water buffaloes calving during the short photoperiod season than those calving in the long photoperiod season (0.4239 vs. 0.2474, $P > 0.000$). The latter only had 0.2645 odds to become pregnant than those calving during the short photoperiod season. The negative effect of long photoperiod was observed indifferently of parity. In conclusion, primiparity and the long photoperiod affected water buffalo cow's reproductive performance, decreasing pregnancy probability during the first 90 days postpartum.

Keywords: Water buffalo cows, anestrus, parity, photoperiod, season, probability of pregnancy.

Probabilidad de preñez antes de los noventa días postparto en búfalas de agua

Resumen. Este estudio tuvo como objetivo determinar el efecto del número de partos y de la época de partos sobre la probabilidad de preñez en los primeros 90 días (90) postparto en búfalas de agua. Se realizó un análisis retrospectivo de los registros reproductivos de 1 465 búfalas de agua (3 181 preñeces). Las búfalas se agruparon según el número de partos en uno, dos o tres y más partos; mientras que la época de partos se creó con los siguientes valores: fotoperiodo largo (marzo-agosto) y fotoperiodo corto (septiembre-febrero). Se calcularon las probabilidades de preñez mediante el modelo de regresión logística de efectos mixtos, y se ajustó un modelo lineal mixto generalizado con intersecciones aleatorias para determinar los odds logarítmicos para la preñez 90 días postparto. La probabilidad de preñez 90 días postparto fue de 0.3645, y fue menor en primíparas (0.2717) en comparación con las búfalas de dos (0.3863) y tres o más partos (0.5166). La probabilidad de preñez 90 días postparto aumentó 1.77 odds por cada aumento en el número de partos. La probabilidad de preñez 90 días postparto fue mayor en las búfalas de agua que parieron durante la época de fotoperiodo corto (0.4239 vs 0.2474, $P > 0.000$), que las que parieron en la época de fotoperiodo largo. Además, las últimas tuvieron solo 0.2645 odds de preñez que aquellas que parieron durante la época de fotoperiodo corto. El efecto negativo del fotoperiodo largo se observó indistintamente del número de partos. En conclusión, la primiparidad y el fotoperíodo prolongado afectaron el desempeño reproductivo de las búfalas de agua, disminuyendo la probabilidad de preñez durante los primeros 90 días postparto.

Palabras clave: Búfalas de agua, anestro, número de partos, fotoperiodo, época, probabilidad de preñez

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Introduction

The profitability of buffalo production systems largely depends on an excellent reproductive efficiency (Shah et al., 1991; Sweers et al., 2014), and to achieve this goal, a calving to conception interval 90 days is necessary. Buffaloes with low reproductive efficiency generate economic losses. Water buffaloes with delayed conception are 24 to 27 % less profitable than those becoming pregnant earlier (Khan et al., 2008), and each extra day of calving interval, above the herd average, generate a decrease in earnings of \$ 1.87 (Safari et al., 2019).

A low reproductive performance implies greater costs by feeding and medicines, more non-productive days, decreased milk yield, less economic return for commercializing non-pregnant buffaloes, decreased number of replacements, males to sale, and sale of potentially productive animals (Shah et al., 1991; Cicek et al., 2017; Khan et al., 2008; Ribeiro et al., 2012; Safari et al., 2019). In this scenario, achieve the goal of getting the buffaloes pregnant 90 days postpartum is a priority, although it can be challenging because several factors affect the reproductive performance of buffaloes (Nava-Trujillo et al., 2020b).

Parity and season of calving affect reproductive performance. Primiparous buffaloes have longer calving to first mount/service, more prolonged calving to conception interval, lower pregnancy rate, and longer calving interval (Hassan et al., 2017; El-Tarabany 2018; Nava-Trujillo et al., 2018; Nava-Trujillo et al., 2019; Nava-Trujillo et al., 2020a). Similarly, long photoperiod season promotes similar effects, and water buffaloes calving during spring and summer have lower reproductive performance than those calving in autumn and winter (Hassan et al., 2017; Ramadan 2018; Nava-Trujillo et al., 2019; Nava-Trujillo et al., 2020a). Realize the postpartum moment when buffaloes become pregnant, and identifying the non-pregnant buffaloes during the early postpartum, is of great interest. It will allow establishing a plan to control the postpartum anestrous period and to shorten the calving to conception interval in high-risk groups to ensure that most buffaloes get pregnant at the correct time. Therefore, this research aimed to determine the probability of water buffaloes became pregnant in the first 90 days postpartum and to establish whether parity and season of calving affected the achievement of this reproductive target.

Materials and Methods

This is a retrospective study analyzing reproductive records of 1 465 crossbred (Murrah/Mediterranean) water buffalo cows from three herds, including a total of 3 181 pregnancies for eight years (2010 – 2018). The farms were located in Jesus María Semprún county, Zulia State, Venezuela, a tropical moist forest, with a mean daily temperature of 28.3 °C, a mean relative humidity of 80 %, and rainfall of 1800-3800 mm/year (Ewel et al., 1976). In the farms, animals grazed in pastures of *Brachiaria humidicola* and *Brachiaria arrecta* in one- to two-day paddock rotations, with access to fresh water and a commercial mineral mix ad libitum. Mechanical milking was performed twice a day after a short time of suckling (1-3 min) 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 dams to remove residual milk from the udder. Buffalo cows were served by natural mount, and an entry water buffalo bull for every 30-40 cows was present 24 hours during the year-round. The farmers kept paper records of each water buffalo cow. Additionally, in each farm, a preventive medicine standard program that included procedures to diagnose, prevent, and treat prevalent diseases and the application of

mandatory vaccines against brucellosis, bovine rabies, clostridial diseases, leptospirosis, and foot and mouth disease was carried out.

Statistical analysis

Information about parity, the month of calving, and pregnancy status at day 90 postpartum were obtained from the records. Variable calving season was created as follows: March-August (long photoperiod) and September-February (short photoperiod). The main predictors were parity (categorized as one, two, or three and more calvings) and calving season. The outcome of interest was a binary variable about the pregnancy status at 90 days postpartum (pregnant or non-pregnant).

A generalized linear mixed model was fitted with random intercepts to the log odds of becoming pregnant 90 days postpartum, which were regressed on parity and season of calving. All analyses were performed in Stata 15.1, and comparisons were adjusted by Sidak's method, in which the significance level to reject the null hypothesis was $1-(1-\alpha)^{1/k}$ (α : familywise error rate, 0.05; k: number of pre-planned

probabilities from the mixed-effects logistic regression model were calculated through the command margins

(Stata 15.1). Differences were considered statistically significant when P values were ≤ 0.05 .

Results

The general probability of pregnancy 90 days postpartum was 0.3645 (95 % CI: 0.3465 - 0.3825), and this probability increased as parity increases: 0.2717 (95 % CI: 0.2500 - 0.2935) for primiparous buffaloes, 0.3863 (95 % CI: 0.3665 - 0.4062) for two-calved buffaloes and 0.5166 (95 % CI: 0.4811 - 0.5521) for buffaloes with ≥ 3 calving ($P < 0.000$). As the number of calving increases, the odds of becoming pregnant in the first 90 days postpartum increased 1.7738 times (95 % CI: 1.5654 times - 2.01 times, $P < 0.000$).

In water buffaloes calving during short photoperiod season probability of pregnancy during the first 90 days postpartum was 0.4239 (95 % CI: 0.4014 - 0.4465),

and it was greater than in buffaloes calving in the long photoperiod season (0.2474; 95 % CI: 0.2197 - 0.2750; $P < 0.000$). The odds of becoming pregnant 90 days postpartum in water buffaloes calving during the long photoperiod season were 0.2645 times (95 % CI: 0.1627 times - 0.4298 times, $P < 0.000$) than in those calving during the short photoperiod season.

The positive effect of increasing parity on the probability of pregnancy 90 days postpartum was observed regardless of calving season. Likewise, the negative effect of calving during the long photoperiod season was observed in all parity categories (Table 1).

Table 1. Effect of parity and calving season on the predicted probability of pregnancy 90 days postpartum in water buffaloes.

Parity	Season			
	Short photoperiod		Long photoperiod	
	Adjusted probability	95 % CI	Adjusted probability	95 % CI
1	0.3315 ^{aA}	0.3020 0.3610	0.1530 ^{aB}	0.1259 0.1802
2	0.4483 ^{bA}	0.4242 0.4725	0.2636 ^{bB}	0.2326 0.2938
3	0.5706 ^{cA}	0.5305 0.6106	0.4094 ^{cB}	0.3446 0.4742

Values from the same column with different lowercase letters are significantly different ($P < 0.05$). Values from the same row with different uppercase letters are significantly different ($P < 0.05$).

Discussion

In this study, parity and calving season affected the probability of buffalo cows became pregnant in the first 90 days postpartum. The probability of pregnancy increased as parity increased, and therefore, primiparous buffaloes had the lowest probability of pregnancy. This likely occurs due to the greater incidence of anestrus observed in this group of buffaloes (Sosa et al., 2016). Jamuna et al. (2015) observed that primiparous buffaloes had a longer interval to first service and conception than multiparous; and it was recently observed that primiparous buffaloes had a more extended anestrus period (30 extra days) and a lower probability of having the first mount before 60 and 100 days postpartum compared with multiparous buffaloes (Nava-Trujillo et al., 2019). Also, the reproductive performance of primiparous buffaloes was related to milk yield, and those with greater milk yield had a lower pregnancy rate, longer calving interval, and lower probability to a calving interval ≥ 400 days (Jamuna and Chakravarty, 2016; Nava-Trujillo et al.,

2020a). Milk yield determines the energy balance, and as primiparous buffaloes are still growing, they develop a more profound negative energy balance, affecting their reproductive performance (Vecchio et al., 2007). A longer calving interval was observed in primiparous buffaloes having their first calving older (Zicarelli 2007), while younger buffaloes to the first calving had a service period 31.85 days shorter (Verma et al., 2018). Additionally, as herds used in this study practice suckling to induce milk letdown before milking, ovarian activity resumption may be delayed (Qureshi and Ahmad, 2008; Rijasnaz et al., 2014; Kantharaja et al., 2018). It seems that primiparous buffaloes are more sensitives to the negative effect of suckling than multiparous buffaloes (El-Fouly et al., 1976).

The reproductive activity of water buffaloes increases during the short photoperiod season (Zicarelli 2009; Gasparrini 2019; Nava-Trujillo et al., 2019), and the long photoperiod negatively affects

reproductive performance, as observed in the present study. The probability of pregnancy 90 days postpartum was lower in buffaloes calving during the long photoperiod season, regardless of parity. This was probably due to the delay in the resumption of ovarian activity and the lower fertility observed during this season, which generated a lengthening of the interval to the first service and conception, respectively. Estrus activity is lower during the long photoperiod season, and only 29.4 % of mounts occurred this season (Nava-Trujillo et al., 2019). Moreover, water buffaloes calving during this season has a lower probability of being mounted during the first 60 and 100 days postpartum than calving during the short photoperiod season (Nava-Trujillo et al., 2019); even hypothalamus, hypophysis, and follicles are less responsive to exogenous hormones during the long photoperiod season (Barile et al., 2015). Lower pregnancy rates were observed during the long photoperiod season (El-Tarabany 2018; Dharap 2016; Qayyum et al., 2018), and a high incidence of embryo mortality has also been reported. Di Francesco et al. (2012) observed 7.3 % of late embryo mortality in autumn and 23 % during the transition to long photoperiod ($P < 0.05$). In Pakistan, a 60 % pregnancy loss was reported between May-July in contrast with

the 33 % observed between September and December (Qayyum et al., 2017). All these findings occur because, in the season of long photoperiod, the corpus luteum receive lower blood flow and produce lower progesterone, affecting embryo development and survival; and smaller embryos have a greater probability of mortality (Campanile et al., 2005; Russo et al., 2010; Di Francesco et al., 2012; Neglia et al., 2012; Balestrieri et al., 2013).

Nevertheless, the effect of the season could be modulated by other factors. It was observed that parity affects calving concentration, and heifers are less seasonal than multiparous (Zicarelli 1994; Borghese et al., 1995; Ceron-Muñoz et al., 2017). The increase in the intake of metabolizable energy, zinc, and calcium and a decrease in crude protein consumption, magnesium, phosphorus, and copper were associated with the onset of the breeding season. (Qureshi et al., 1999a). In addition, in Colombia, Sánchez et al. (2017) observed a higher frequency of calving and conceptions in months with a decrease of daily temperature and relative humidity and increased rainfall. In contrast, Sertu et al. (2012) suggested that the supply of forage is a factor involved in the reproductive seasonality of the species.

Conclusions

The present study results showed that primiparous buffaloes and those calving during the season of long photoperiod have a high risk of reproductive failure, it is a lower probability of becoming pregnant 90 days postpartum. Therefore, based on these results, it is

recommended to establish a plan including nutritional, management, genetic, and hormonal strategies to cope with the harmful effects of the first calving and the long photoperiod to improve the reproductive performance of water buffalo cows.

Animal use and care: In this study, data collection did no required interactions with animals.

Conflict of interests: The authors disclose no conflict of interest.

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