

**Impact of calving order, milk production and somatic cell count on the
reproduction of Holstein cows**

SUPPLEMENTARY FILE

Material and methods

Dairy herds

The herds were distributed across 10 out of the 19 municipalities within the Campos Gerais Region of Paraná State, Brazil. The municipalities where the herds were located include Castro, Carambeí, Fernandes Pinheiro, Imbituva, Irati, Ponta Grossa, Prudentópolis, Teixeira Soares, Tibagi, and Witmarsum. The Campos Gerais Region spans an area of 11,761.41 km² and is geographically located between 23°45' and 26°15' south latitude and 49°15' and 50°45' west longitude. The prevailing climate in the region is classified as Cfb according to the Köppen classification, with a smaller portion characterized as Cfa, varying depending on the location. The average minimum and maximum temperatures recorded are 15°C and 25°C, respectively. Moreover, the region experiences an average annual precipitation of 92 mm.

Data collection

The interval before and after artificial insemination (AI) was established by calculating the herds' mean somatic cell count (SCC) and milk production. The aim was to evaluate the percentage of pregnant cows for the two extremes (minimum and maximum) of SCC and production. Pregnancy diagnosis was conducted on some farms using rectal palpation or transrectal ultrasonography. The dairy cows' milk production and composition records were collected during official milk recordings performed at the end of lactation. The data for this analysis were obtained through test-day recordings performed 10 days before and after the AI date. Records of multiparous cows with up to

five lactations were included, and data from cows with fewer than seven milk recordings were eliminated. The milk production records near positive and negative pregnancies were obtained by weighing the milk using the milking equipment on the studied farms.

Milk production and composition

The Parana Association of Holstein Breeders (APCBRH) conducted the milk composition and SCC analyses. Milk protein, fat, and urea nitrogen were measured through infrared spectrophotometry (B 2300 Combi, Bentley), while somatic cell counting was performed using flow cytometry (Somacount 500).

Statistical analysis

The data were analyzed by multivariate logistic regression using the GLIMMIX procedure of the SAS package for Windows- Version 9.3 (SAS Institute Inc., Cary, NC, 2014). The independent variables considered in the model were the classes of primiparous and multiparous cows and the effects of SP, S/C, milk production/cow/day, SCC, fat, protein, and MUN contents, adopting a level of significance of 5%.

Considering that Y_i , $I = 1, 2, \dots$, where i is the variable measured, $Y_i \sim \text{Bernoulli}(\pi)$, and π is the probability of being primiparous or multiparous, this probability function of Y_i can be represented by the model:

$$P(Y_i = y_i) = \pi^{y_i}(1-\pi)^{1 - y_i}, y_i = 0, 1.$$

The final statistical model in the two stages of analysis was obtained by sequentially removing the explanatory variables based on Wald's statistical criterion, applying a cut-off of $p > 0.2$. The explanatory variables incorporated in the final model were considered in order to estimate statistical differentiation between SCC and milk production classes, adopting a level of significance of 5%.

Results and Discussion

The low SCC of the two animal categories indicated that herds with good udder health were kept under adequate hygiene conditions in the facilities and the milking room. The mean SCC analyzed close to AI with adverse pregnancy was low. Thus, factors other than SCC may be compromising the herd's reproductive efficiency (Table 1). Although the SCC is a valid trait for assessing the inflammatory state of the mammary gland, it does not provide information about individual cell populations. The cells that make up the SCC of cow's milk comprise leukocytes, lymphocytes and macrophages, which reflect the animal's inflammatory response (Stocco *et al.*, 2020). However, studies have shown that epithelial cells can account for 25% of the total SCC, while defense cells account for 75% (Sharma; Singh; Bhadwal, 2011). Thus, the variation in the proportion of epithelial cells can determine SCC's true impact on mastitis and milk production. This situation would apply more to older cows because desquamation of the epithelium is more significant in this category (Stocco *et al.*, 2020).

Several factors related to herd management may be associated with a higher pregnancy percentage in multiparous cows. These factors include the dominance of older cows, the absence of hormonal protocols in first-calving cows, increased use of sexed semen in primiparous cows, and the more significant challenges to which primiparous cows are exposed during the postpartum period. As a result, first-calving cows are less affected by metabolic and reproductive diseases during the puerperium, and their care is often overlooked within this context.

Concerning the management of primiparous and multiparous cows aimed at increasing reproductive efficiency, producers understand the importance of making timely and accurate intervention decisions regarding calf survival; however, the cow can also be affected by these interventions, which can have long-term effects. For example, after 1.5 hours of labor, each 30-minute delay in calving assistance can result in a 6-day

longer interval to the subsequent pregnancy (Daly, 2020). Therefore, dystocia during calving can also influence cow reproduction in future pregnancy. This parameter has not been considered herein. However, the most effective approach to prevent losses and problems associated with dystocia is to manage animals in such a way as to reduce their occurrence since this condition affects production, fertility, and the morbidity mortality of cows and calves (Santos, 2021).

It is widely recognized that attention must be paid to the age at first calving (AFC) of heifers, with a recommended age of 23 to 24.5 months for Holstein cows. A reduction in the AFC of heifers was associated with reduced milk production in first lactation and increased dystocia at calving (Gaafar *et al.*, 2011), commonly occurring when heifers calve at less than 22 months of age. Mellado *et al.* (2019) evaluated the impact of AFC and climate conditions at calving on peripartum disorders and reproductive performance of Holstein heifers in a hot environment. The authors observed a negative effect of late AFC (> 26 months) of heifers in a hot environment on dystocic delivery, pregnancy, conception, and culling rates. However, heifers under 2 years at first calving exhibited a higher incidence of metritis and ovarian cysts. The authors thus concluded that milk producers using intensive systems could reduce the AFC by performing earlier insemination without compromising calving assistance and reproductive performance to reduce total rearing costs.

References

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