Journal of Dairy Research

cambridge.org/dar

Impact of parity number, milk production and somatic cell count on the reproduction of Holstein cows‡

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Research Article

‡This article has been updated since its original publication. A notice detailing this change can be found here: https://doi.org/10.1017/S0022029924000177

Cite this article: Barbosa BL, Pedrosa VB, da Silva Leal Karolewski L, de Godoi CC and de Souza Martins A (2023). Impact of parity number, milk production and somatic cell count on the reproduction of Holstein cows. *Journal of Dairy Research* **90**, 343–346. https://doi.org/10.1017/S002202992300078X

Received: 12 March 2023
Revised: 22 November 2023
Accepted: 28 November 2023

Keywords:
Lactation; milk production; multiparous; pregnancy; primiparous cows

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Abstract

This research communication describes the reproductive and productive parameters and somatic cell count (SCC) of primiparous and multiparous cows from specialized dairy Holstein herds in South region of Brazil, and correlates these parameters using test-day records. A total of 24 011 records of animals from 75 producers obtained between 2017 and 2018 were used. The variables analyzed included milk production, SCC, milk fat, protein and urea nitrogen contents and reproductive characteristics (number of services per conception, service period and percentage of pregnant cows). Data were analyzed using multivariate logistic regression and the statistical differentiation between the classes of SCC and milk production. Milk production, SCC, number of services per conception and service period were all higher in multiparous cows. The percentage of pregnant cows was higher when the SCC was less than 200 000 cells/ml and was higher in primiparous cows. There was no adverse effect of milk production on reproductive parameters in high producing cows (>40 kg milk/d), however, the percentage of pregnant cows with this production level was significantly higher in multiparous cows. In conclusion, the milk production level need not affect the reproduction of specialized dairy cows if the animals are kept under adequate environmental conditions.

Over the last several decades, genetic improvement has permitted increases in milk production but at the same time high producing dairy cows have lost reproductive efficiency, measured by ability to rebreed for annual lactation cycles. The causes include impaired reproductive physiology, nutrition and management (Walsh *et al.*, 2011; Gross and Bruckmaier, 2019) and mastitis. The reproduction of cows interferes with the volume of milk produced and with production costs since compromised reproductive performance of the herd will reduce the production of heifers for replacement and increase dry cow maintenance costs. Additionally, pregnancy loss and mastitis are health problems that affect profitability of dairy farms (Dahl *et al.*, 2018).

The number of calvings affects milk production and reproduction. Primiparous lactating cows generally exhibit a more severe negative energy balance than multiparous cows since, in addition to the energy requirements for lactation, they have a lower dry matter intake and require energy for body growth. These factors also compromise their reproductive performance (Walsh *et al.*, 2011). On the other hand, high parity is considered a risk factor associated with clinical and subclinical mastitis, triggering an increase in the somatic cell count (SCC). In addition to greater exposure to pathogens during productive life, old animals are more likely to become infected or develop chronic mastitis. The immune dysregulation process is accelerated due to long-term exposure to stressors such as previous infections, metabolic disorders, heat stress and strain related to high productivity (Cardozo *et al.*, 2015). Mastitis and reproductive problems are the main reasons for voluntary culling of dairy cows. The high costs associated with mastitis and the losses caused by low reproductive efficiency highlight the need for studies that assess the limit of the relationship between SCC and reproduction, whilst taking into account parity number.

The objectives of this study were to analyze the impact of milk production, SCC and parity number on the reproductive performance of cows using test-day records. The hypotheses were that knowledge of the impact of SCC on the reproductive efficiency of primiparous and multiparous cows permits better definition of preventive management strategies and the ability to focus on the reproduction of the most susceptible category of the herd, whilst analysis of the effect of milk production on reproduction of primiparous and multiparous cows will identify

the animal category that is most susceptible to a reduction in reproductive rates due to high milk production.

Material and methods

Dairy herds and data collection

We collected data from specialized dairy Holstein herds of 75 milk farms, totaling 24 011 records of lactating cows obtained between 2017 and 2018, from southern Brazil. The reproductive, production and milk composition parameters were analyzed over 10 d before and 10 d after successful artificial insemination (AI) resulting in confirmed pregnancy (succesful pregnancy: SPR). Likewise, the data were analyzed over the same period when AI did not result in pregnancy (failed pregnancy, FPR). Pregnancy diagnosis was carried out in most farms through ultrasound, every 21 or 30 d, in all inseminated animals that required pregnancy confirmation. In some farms, the diagnosis was made through milk analysis whislt in a few others the natural estrus return was used to initiate repeat breeding. Repeated AI measurements in the one animal were made, and the number of failed AI per animal was recorded.

The production and reproductive characteristics (number of services per conception, service period and percentage of SPR and FPR) were analyzed. Two interval classes of somatic cell count (SCC) were defined for comparative analysis of the percentage of pregnant cows: ⩽200 000 cells/ml and ⩾500 000 cells/ml. For milk production, the groups were ⩽25 and ⩾40 kg/cow/d.

Milk production and composition

The herds were milked with automated equipment. The milking equipments incorporated individual milk sampling capability with the piped system. Levels of fat, protein, milk urea nitrogen and somatic cell score were determined in milk samples.

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 services per conception, service period, milk production/cow/d, SCC, stage of lactation (days in milk), fat, protein, and MUN contents, adopting a level of significance of 5%. The data were considered to show a Bernoulli distribution (binary variables) and model details are given in the online Supplementary file. 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%. The reproductive variable was assumed to be independent of the percentage of pregnant cows.

Results and discussion

On these specialized herds with genetically improved cows the mean milk production of primiparous ($n = 3312$) and multiparous ($n = 5305$) cows was 33.9 and 36.8 kg/d, respectively, and the mean SCC values were 162 and 347 × 1000 cell/ml, respectively. Multiparous cows produced more milk and had a higher SCC ($P < 0.05$). The SCC increases with increasing calvings because of the greater replacement of epithelial cells and hence loss into milk, in addition to the higher rate of infection of the mammary gland. The activation of the immune system in young cows at the beginning of lactation is mostly beneficial and is related to an effective immune response against stress conditions (Stocco *et al.*, 2022). Although the SCC found here was low, it may compromise milk production. Chen *et al.* (2021) estimated daily milk loss of 0.19 kg from primiparous cows with SCC between 100 and 200 000 cells/ml, and from multiparous the milk loss was 0.34 kg.

The effects of the relationship between milk production and composition with reproduction were analyzed in primiparous and multiparous cows (Table 1). The services per conception values found here were low, indicating high reproductive efficiency of the herds. The service period was similar between categories, corresponding to a calving interval of 14 and 15 months for primiparous and multiparous cows, respectively. If one considers that this does represent a negative impact on the calving interval, there are two potential explanations for it: high-producing dairy cows may lack sufficient blood estradiol concentrations to induce estrus and ovulation (Sauls *et al.*, 2016), and many producers in the region used bovine somatotropin, which directs nutrients from the body to the mammary gland and maintains the lactation curve stable for a longer time. Whether this second factor is important depends on management practices, as some producers will delay somatotropin use until pregnancy is confirmed. Historically, the literature recommends a service period of up to 100 d and a calving interval of 12 months, but the values found here are acceptable since they were obtained for highly productive animals.

Milk production recorded during AI that resulted in FPR was similar to the production during AI with SPR (Table 1),

Table 1. Number of services per conception (S/C), service period (SP), mean milk production (MP), somatic cell count (SCC), and milk fat, protein and urea nitrogen (MUN) contents of milk recorded on test-days closest to artificial insemination (AI) that resulted in success or failure pregnancy of primiparous and multiparous cows belonging to dairy herds in southern Brazil

Category (n)	Pregnancy	S/C	SP (days)	MP (kg/cow/d)[1]	SCC (× 1000 cells/ml)	Fat (g/100 g)	Protein (g/100 g)	MUN (mg/dl)
Primiparous (1,508)	Success	1.28b	139	35.14b	129b	3.70	3.20	10.5
Multiparous (1,660)	Success	1.69a	150	39.04a	239a	3.70	3.30	10.0
Primiparous (1,920)	Failure	–	–	35.7b	147b	3.76	3.25a	11.63
Multiparous (3,545)	Failure	–	–	40.5a	250[a]	3.71	3.20b	11.93

Means in the same column within success or failure pregnancy followed by different lowercase letters differ significantly ($P < 0.05$) by the Tukey test. [1]Last lactation with at least 7 test-day milk records during lactation. Primiparous: one lactation. Multiparous: two to five lactations. Test-day milk records obtained within a period of 10 d before and 10 d after the date of AI. n: number of animals.

https://doi.org/10.1017/S002202992300078X Published online by Cambridge University Press

indicating that cows became pregnant even if they produced high milk volumes. Milk production close to AI with SPR was higher among multiparous cows. Milk production is the most frequently used trait in dairy cow selection programs, even though increased production is related to a decline in reproductive (rebreeding) efficiency. Although failure to rebreed has been associated with high milk production for many years, its cause is multifactorial and cannot be attributed to production alone. Improvements in dairy farms include management practices, a healthy environment and enhanced nutrition.

The mean SCC of cows obtained close to AI that resulted in FPR was similar to the count close to AI that resulted in SPR (Table 1). Thus, the low SCC did not seem to interfere with cow fertility. Similarly, this parameter did not compromise milk production in both categories. The SCC was significantly higher in multiparous cows compared to primiparous cows for AI which resulted in both successful and failure pregnancies. Cows with clinical mastitis at the beginning of lactation exhibit a longer service period and a larger number of services per conception (Salar *et al.*, 2019). However, Dahl *et al.* (2018) evaluating the effects of clinical and subclinical mastitis on pregnancy losses im primiparous cattle, observed that subclinical mastitis may not cause a sufficient inflammatory response to compromise follicular growth or oocyte quality.

A fat/protein ratio lower than 1.0 or higher than 1.5 may indicate metabolic diseases in the herd, such as ruminal acidosis or ketosis, respectively (Bostanova, 2021). These diseases can also compromise cow fertility. The fat and protein contents recorded close to AI that resulted in both SPR and FPR were similar (Table 1). These isolated components were not related to the reproductive performance of cows.

There was an association between SCC and reproduction. The average pregnancy percentage was only 7.6% in cows with a SCC >500 000 cells/ml compared to the average of 82.4% obtained for cows with a SCC <200 000 cells/ml (Fig. 1). Among cows with a SCC ⩽200 000 cells/ml, a higher pregnancy percentage was observed in primiparous animals than in multiparous. The opposite occurred among cows with a SCC ⩾500 000 cells/ml, with a higher percentage of pregnant multiparous compared to primiparous cows, but overall, parity number did significantly ($P < 0.05$) influence the reproductive parameters of dairy cows. Regardless of the SCC level, cows that did not get pregnant in the first service returned to the group to be inseminated again. Cows that failed at first service were returned for further services, independent of SCC level. Thus, reproductive performance of primiparous cows was apparently more compromised when SCC was high. Increases in SCC are usually associated with an increase in defense cells to fight off an invading pathogen. During inflammation in the mammary gland, leukocytes in the blood are increased, producing pro-inflammatory cytokines that can affect follicular fluid, oocyte development and expression of specific genes in oocytes, thereby compromising reproduction (Bilodeau-Goesseels, 2003). According to Wolfenson *et al.* (2016), subclinical mastitis compromises the functioning of the pre-ovulatory follicle, resulting in low fertility. The authors highlighted that the main effect was a delay in ovulation and also the low production of follicles, verified in approximately 1/3 of cows with subclinical mastitis.

Primiparous cows typically have a higher concentration of non-esterified fatty acids and ketone bodies after calving comparing to multiparous, which is believed to be related to decreased imune function, incidence of uterine diseases and delayed ovulation. Therefore, under high SCC condition, such as in the present study, this effect may have been pronouced, further compromising the fertility of primiparous cows more than multiparous. Additionally primiparous are better able to mantein adequate body temperature under heat stress conditions anda have lower SCC. However, when they are challenged by high SCC, their physiological condition is challenged, compromising fertility.

There was no difference ($P > 0.05$) in the pregnancy percentage between primiparous and multiparous cows with production levels of up to 25 kg/cow/d. However, in the higher yielding category, multiparous cows exhibited better reproductive performance.

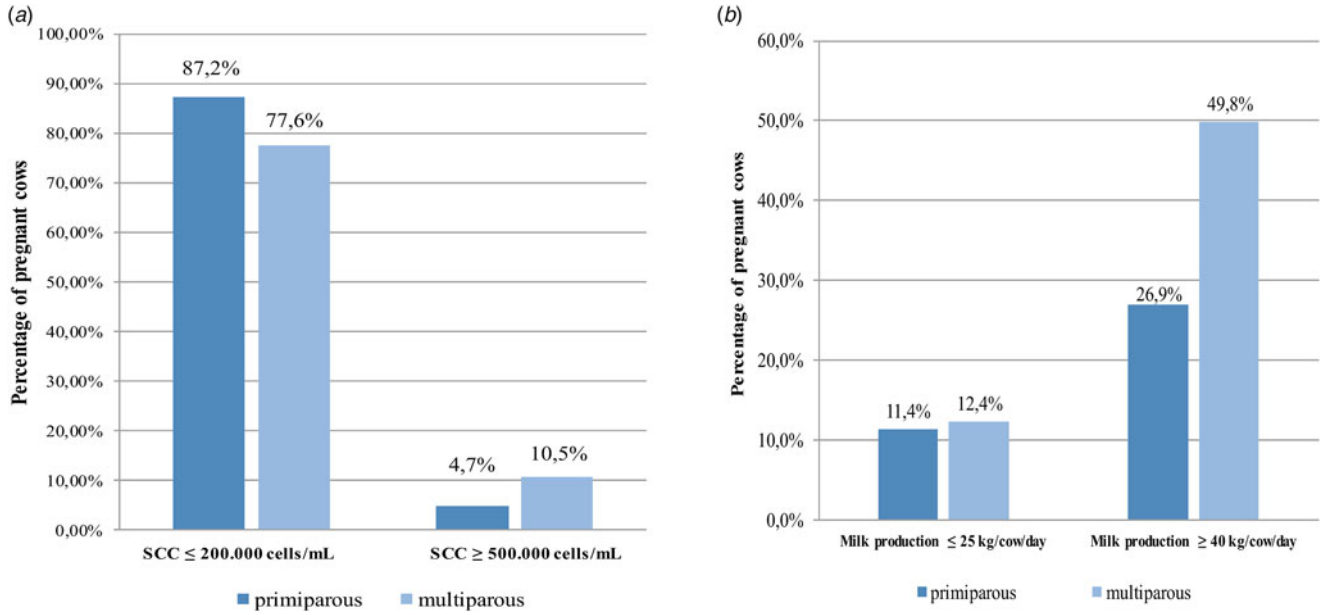

Figure 1. Percentage of pregnant cows (primiparous and multiparous) for two SCC intervals (⩽200 000 cells/ml and ⩾500 000 cells/ml) (B) and two milk production levels (⩽25 kg/cow/d and ⩾40 kg/cow/d) (B) in southern Brazil. Values of n as follows: Low SCC, primiparous 1,315 and multiparous 1,288. High SCC, primiparous 71 and multiparous 174. Low production, primiparous 172 and multiparous 205. High production, primiparous 405 and multiparous 827.

https://doi.org/10.1017/S002202992300078X Published online by Cambridge University Press

One possible explanation is their lower growth requirements, so that nutrients can be directed towards maintenance, production and reproduction. The more important point is that higher milk production was not associated with lower pregnancy outcome in either age category, indicating that factors other than production interfered with the fertility of cows.

In conclusion, high milk production does not necessarily negatively affect the reproduction of cows. Primiparous cows were apparently more affected by the impact of SCC on reproduction than multiparous, with a higher pregnancy percentage among primiparous cows with a SCC ⩽200 000 cells/ml. Cows with two or more calvings exhibited better reproductive performance even when daily milk production was high (⩾40 L/cow/d). Therefore, primiparous had a lower pregnancy rate under high milk production than multiparous, showing that this category is more sensitive to high production, perhaps as they are still growing such that energy defecit makes a greater contribution to impaired reproduction.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S002202992300078X.

Acknowledgements. This study comprised the Master's Thesis of the first author. It was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors thank the Frísia Cooperativa Agroindustrial, that supports the work by providing the data.

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