

Association between the perineal hygiene score at calving, puerperal uterine disorders, and reproductive efficiency in crossbreed dairy cows

Relação entre escore de higiene perineal no parto, distúrbios uterinos puerperais e eficiência reprodutiva de vacas leiteiras mestiças

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Highlights

High dirtiness perineal scores are associated with uterine puerperal disorders.
Reproductive efficiency is not associate with perineal dirtiness score at calving.
Cows present similar perineal dirtiness scores at pastures and compost barn system.

Abstract

Retained placenta, metritis, and clinical endometritis are prevalent diseases on dairy farms; they lead to economic losses and reduce the longevity of dairy cows in dairy production systems. Hence, it is important to investigate potential predisposing factors. The aim of this study was to evaluate the relationship between perineal hygiene during calving and the incidence of retained placenta, metritis, and clinical endometritis, and their effect on reproductive efficiency in crossbred dairy cows. At two farms, pre-calving cows (n = 101) were evaluated for perineal dirtiness before calving by visual observation. They received a score of 1, free of feces and a dry perineum; a score of 2, slight presence of feces and a moist perineum; or a score of 3, a mild-wet perineum covered by feces (over 10% of the surface). After calving, cows were subjected to visual observation and gynecological evaluation to identify retained placenta, metritis, and clinical endometritis. Cows with a hygiene score of 2 or 3 showed higher rates of retained placenta (41.9% vs. 14.0%, P = 0.0049), metritis (32.5% vs. 14.0%, P = 0.0191), and clinical endometritis (42.5% vs. 15.8%, P = 0.0061) compared with cows with a score of 1. There were no effects

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on reproductive efficiency. The findings indicate that the degree of perineal dirtiness in prepartum crossbred dairy cows is associated with the occurrence of uterine puerperal disorders.

Key words: Dairy cows. Reproductive diseases. Reproductive rates. Calving hygiene. Reproduction.

Resumo

Retenção de placenta, metrite e endometrite clínica são distúrbios presentes nas fazendas leiteiras, levando a perdas econômicas e reduzindo a longevidade das vacas nos sistemas de produção, dessa forma, é importante investigar os possíveis fatores predisponentes. Objetivou-se avaliar a relação entre higiene perineal antes do parto e a ocorrência de retenção de placenta, metrite, endometrite clínica e sua influência na eficiência reprodutiva de vacas leiteiras mestiças. Em duas propriedades, vacas no pré-parto (n=101) foram avaliadas quanto ao grau de sujidade perineal antes do parto, por observação visual e classificadas em escore 1 - livre de fezes e períneo seco; escore 2 - presença sutil de fezes e períneo úmido; escore 3 - períneo moderadamente molhado, coberto por fezes (acima de 10% da superfície). Após o parto, as vacas foram avaliadas por observação visual e avaliação ginecológica para diagnosticar retenção de placenta, presença de metrite e endometrite clínica. Vacas com escores de higiene 2 ou 3 tiveram maior incidência de retenção de placenta (41,9% x 14,0%, $P = 0,0049$), metrite (32,5% x 14,0%, $P = 0,0191$) e endometrite clínica (42,5% x 15,8%, $P = 0,0061$) se comparadas a vacas com escore 1. Não foram observados efeitos na eficiência reprodutiva. Esses achados indicam que o grau de sujidade perineal de vacas leiteiras mestiças no pré-parto está relacionado com a ocorrência de desordens uterinas pós-parto.

Palavras-chave: Bovinos leiteiros. Doenças reprodutivas. Índices reprodutivos. Higiene no parto. Reprodução.

Introduction

Reproductive efficiency is highly influential on dairy farm profitability. A cow's productive life is related to the age at first calving and the calving interval, in addition to health events that occur during the postpartum period (Bellows et al., 2002; Fernandes et al., 2001). Pre- and post-partum hygiene in dairy cows can be neglected; however, they are factors associated with the development of disorders in the post-partum period and the recovery of ill animals (Kronfeld et al., 2022). Understanding the mechanisms involved in uterine diseases is an important part of breeding programs, but prevention of these diseases during the postpartum period remains uncertain (Sheldon & Dobson, 2004). For several years it had been assumed

that the uterine environment was sterile (Semambo et al., 1991). Nevertheless, it is known that nonpathogenic bacterial populations do exist and maintain the balance of the uterine environment and provide protection. Physiologically, the cervix behaves as a barrier that prevents external bacteria from entering the uterus. During parturition, those barriers are breached, and vaginal and external bacteria can infect the uterus. Moreover, during fetal expulsion, uterine and vaginal fluids may mix (Miranda-CasoLuengo et al., 2019). Within the first 2 weeks postpartum, a wide variety of bacteria can be detected in 90% of production cows (Sheldon, 2014; Walker et al., 2015).

Retained placenta and uterine diseases including metritis and endometritis

are prevalent in dairy cows (Djuricic et al., 2012). Severe postpartum metritis is diagnosed in cows exhibiting an enlarged uterus, a reddish-brown discharge with a foul odor, fever, and systemic signs of illness within 10 days of lactation. For decades, the incidence of uterine metritis has been constant in dairy cows at around 40%, and there are several associated factors (Sheldon & Dobson, 2004). At present, the incidence of metritis, endometritis, and retained placenta in herds is 11%-36% (Corrêa et al., 2014).

Clinical endometritis is characterized by purulent material and detectable vaginal discharge for 21 days or longer after calving; it occurs in 10%-20% of cows, according to breed, country, and herds (Leblanc et al., 2002; Potter et al., 2010). Subclinical endometritis, defined as endometrial inflammation in the absence of systemic signs, appears as a result of bacterial contamination in the uterine lumen and inflow of polymorphonuclear leukocytes, which are drawn to the uterus due to chymosin synthesis and play a key role in uterine immune response (Sheldon et al., 2006).

Studies have demonstrated the relationship between animals experiencing disorders during the postpartum period and health and reproductive efficiency. These issues include extending calving to conception (up to 24 days longer than healthy cows in average), a higher insemination number per pregnancy, an important indicator for reproductive control, and cows with retained placenta and metritis present a conception rate up to 14% lower (Husnain et al., 2023; Ramos et al., 2022). Along with effects on reproductive performance, postpartum diseases in dairy herds lead to a reduction in milk yield, low fertility, increased treatment costs, and negative yields (McDougall, 2001). Based on

knowledge of different factors that might contribute to postpartum uterine disorders as well as reproductive efficiency, the aim of this study was to evaluate the association between perineal hygiene at calving and the occurrence of retained placenta, metritis, and clinical endometritis, and the influence of these conditions on reproductive efficiency in crossbred dairy cows.

Material and Methods

Location, animals, and management

The data from two dairy farms located in Triângulo Mineiro, Minas Gerais, Brazil, were collected. One of the farms has on average of 737 lactating dairy cows producing 17,500 L of milk per day from three daily milkings, and the other farm has on average of 360 lactating dairy cows producing an average of 7,800 L of milk per day from three daily milking. The herds of both farms comprise Girolando cows (Holstein × Gyr crossbreed).

Farm 1 has a semi-confined production system. During summer, the cows remain on pasture and are supplemented with concentrate and roughage provided in covered feeders. During winter, 100% of feed is offered in covered feeders. At the time of evaluation, the average maximum temperature was 23.4 °C and there was only 0.27 mm of rain. For farm 2, lactating dairy cows are kept in a compost barn system with covered feeders and receive a total mixed ration (TMR) composed of corn silage, concentrate, and mineral mixture twice a day. The average maximum temperature during the months of January to July was 22.1 °C and there was 0.29 mm of rain during this period. All cows had ad libitum access to water, and

the diet was formulated in accordance with the National Research Council [NRC] (2001). On both farms, the animals were treated with bovine somatotropin (bST; Lactotropin®, Agener União, Brazil) starting 60 days after calving; it was suspended at the moment that the cow started to produce < 15 L of milk per day or when the cow reached 6 months of pregnancy.

Data and parameters evaluated

Data from farm 1 were collected in January and February 2020 and data from Farm 2 were collected from January to July 2020. The evaluated animals were in the prepartum lot, in a maternity barn, and were

clinically healthy. The cows were analyzed by the same evaluator on the day of calving (day 0) at each farm because the distance between them was small. From 101 cows evaluated, only 93 presented normal calving and were used for the analyses; the eight excluded cows presented dystocia.

The perineal hygiene score was determined based on evaluation of perineal hygiene within 12 hours before calving, namely: 1, a completely dry perineum free of feces (Figure 1); 2, a moist perineum with a slight presence of feces on 1%-10% of the perineal area (Figure 2); or 3, a moderately wet perineum with over 10% of the perineal area covered with feces (Figure 3) (Schuenemann, 2010).

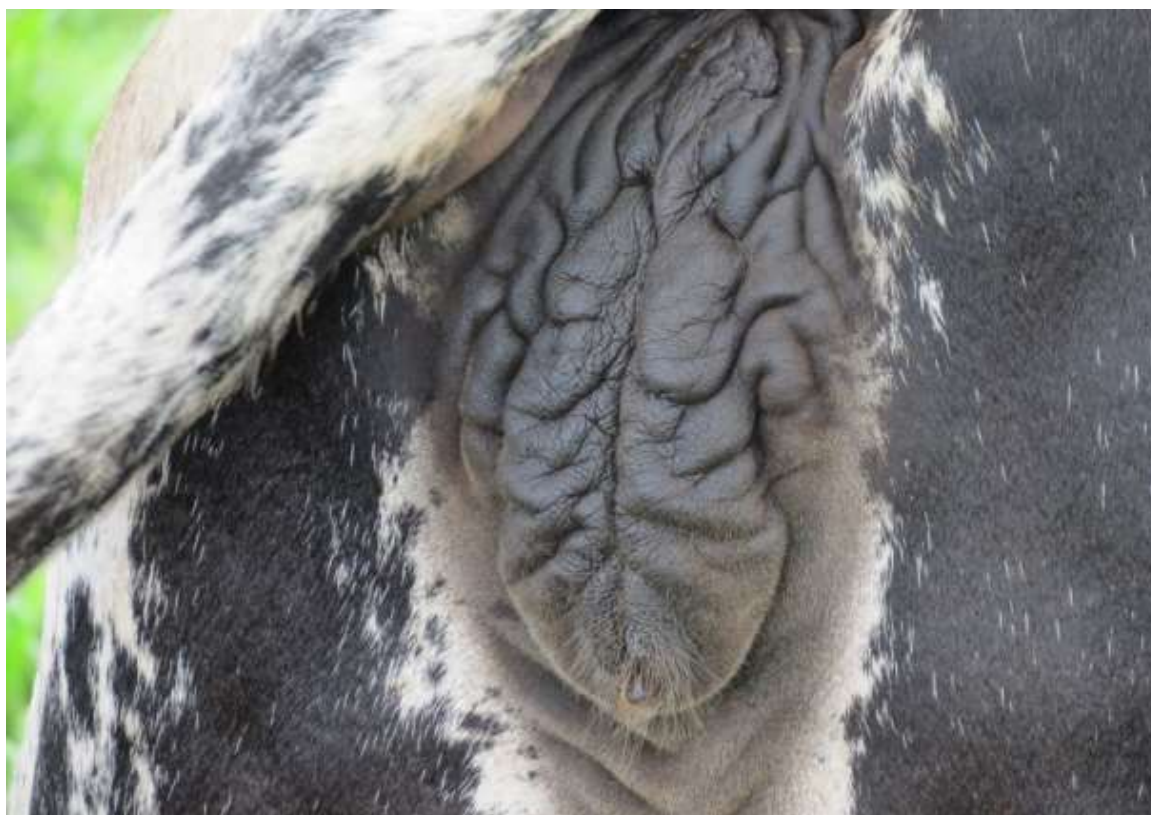


Figure 1. A perineal hygiene score of 1: The perineal area is completely dry and free of feces (this image is part of a personal archive).

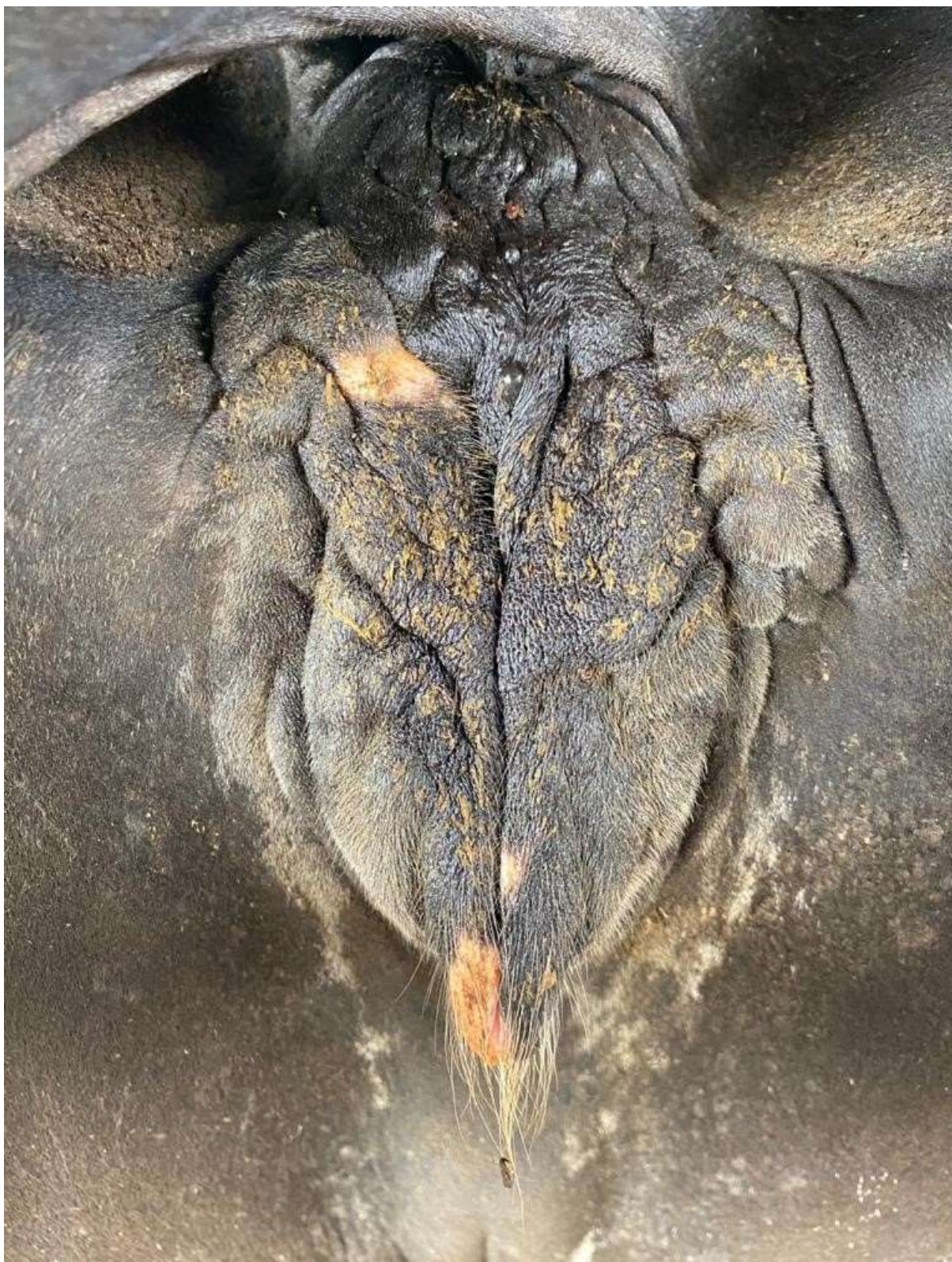


Figure 2. A perineal hygiene score of 2: There is a wet perineum, and feces is present on 1%-10% of the perineal area (this image is part of a personal archive).

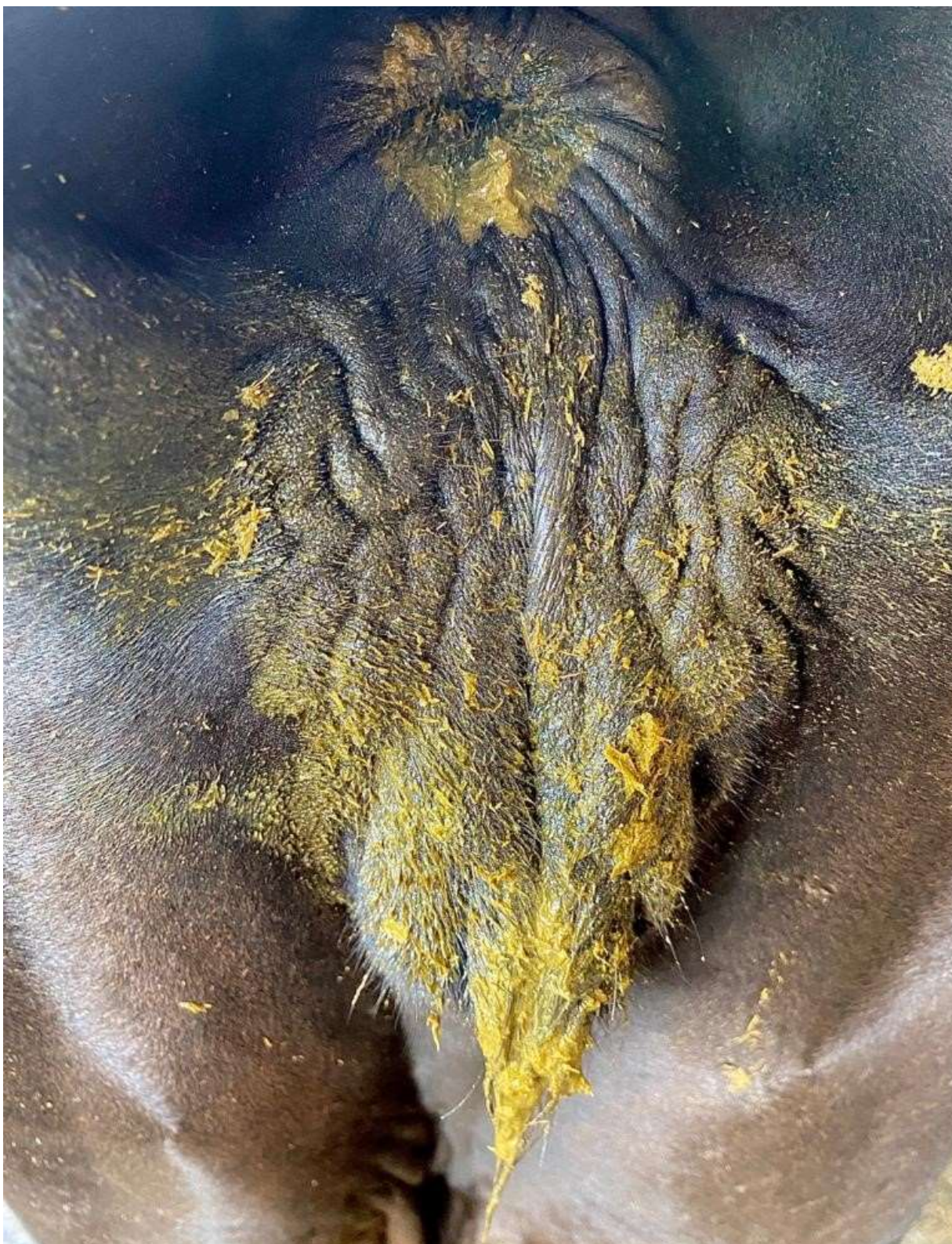


Figure 3. A perineal hygiene score of 3: There is a moderately wet perineum, with > 10% of the perineal area covered with feces (this image is part of a personal archive).

Diagnosis of puerperal diseases and reproduction management

After calving, cows that failed to eliminate the fetal membranes within 12 hours were diagnosed with retained placenta. All newly calved cows were submitted to gynecological evaluations every 15 days, with ultrasonography and vaginal discharge evaluated by Metrichex® (Simcro Ltda., Port Alegre - RS, Brazil) to verify the presence of metritis during the first 21 days after calving and clinical endometritis within 22-35 days after calving.

According to Sheldon et al. (2006), metritis is an abnormally enlarged uterus with fetid watery red-brown uterine discharge; it is associated with signs of systemic illness and fever ($> 39.5^{\circ}\text{C}$) up to 21 days after parturition. Cows diagnosed with metritis were submitted to systemic antibiotic treatment and included in reproductive management only after recovery. Clinical endometritis was defined by the presence of purulent vaginal discharge containing more than 50% pus, as analyzed by Metrichex®, an involuted uterus upon transrectal palpation, and no clinical systemic signs diagnosed between 22 and 35 days postpartum (Sheldon et al., 2009).

The reproductive management of animals was similar on both farms, with fixed timed artificial insemination protocol (FTAI) performed every 15 days. The pregnancy diagnosis by ultrasonography was conducted 30 days after FTAI.

Statistical analysis

All data the perineal hygiene score; diagnosis of retained placenta, metritis,

and clinical endometritis; and the evaluated indices were recorded in a Microsoft Excel worksheet (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed with SAS Studio (SAS Institute Inc., Cary, NC, USA). The univariate procedure was used to determine whether the data had a normal distribution; normality was also assessed visually by using the Kolmogorov-Smirnov method. The number of artificial inseminations per conception and days open were used as dependent variables and estimated via analysis of variance (ANOVA) by a generalized linear mixed model adjusted by the GLIMMIX procedure with a Gaussian distribution; farm was included as a random effect and the perineal hygiene score ($1 \times 2 + 3$) was included as the independent variable. A generalized linear mixed model with the GLIMMIX procedure was used to evaluate the occurrence of retained placenta, metritis, and clinical endometritis; conception; and culling as dependent binary variables with logistic regression; property was a random effect and the perineal hygiene score ($1 \times 2 + 3$) was the independent variable. Only variables with $P \leq 0.15$ were retained in the final model. Statistical significance was set as $P \leq 0.05$ for the main effects and interactions.

Results and Discussion

In the present study, 101 parturitions were evaluated. Ninety-three cows with eutocia were classified according to the perineal hygiene score; the distribution was 50 cows (53.76%) with a score of 1, 33 cows (35.48%) with a score of 2, and 10 cows (10.75%) with a score of 3. Because there were very few animals with a score of 3, the data from animals assigned a score of 2 or 3

were combined and were analyzed together. There were no differences in the hygiene score distribution between the farms ($P \geq 0.15$). The incidence of retained placenta was more prevalent in cows with a perineal hygiene score of 2 or 3 (41.9%) compared with cows with a perineal hygiene score of 1 (14%) ($P = 0.0049$). The incidence of metritis (14%) and clinical endometritis (15.8%) was lower in cows with a perineal hygiene score of 1 compared with cows with a score of 2 or 3 (metritis = 32.5%, $P = 0.0191$; clinical endometritis = 42.2%, $P = 0.0061$) (Table 1). Schuenemann (2010) evaluated the levels of dirtiness of the perineal region of Holstein cows and reported a higher incidence of metritis for cows with a dirty perineal region

compared with cows that during calving presented a perineal region free of feces and moisture. Sheldon (2014) reinforced that the cleanliness of calving sites is related to the postpartum development of uterine disorders. This relationship may be due to the angle between the vulva and the anus of cows, leading to fecal contamination of the vagina. Potter et al. (2010) stated that hygiene gaps are among the risk factors responsible for the development of uterine diseases. However, the authors reported that hygiene markers including fecal consistency, the cow cleanliness score, and disinfection of calving equipment were not related to the development of uterine diseases, differently from the findings of the present study.

Table 1

Influence of the perineal hygiene score on the incidence of retained placenta, metritis, and clinical endometritis in crossbred dairy cows in the Triângulo Mineiro region, 2020

Perineal score (n)	Retained placenta $P = 0.0049$	Metritis $P = 0.0191$	Clinical endometritis $P = 0.0061$
1 (50)	14.0% ^b	14.0% ^b	15.8 % ^b
2+3 (43)	41.9% ^a	32.5% ^a	42.2 % ^a

n - number; a, b - different letters between lines indicate a significant difference ($P < 0.05$).

Poor environmental hygiene may increase the risk of infection, and there is evidence of potential immunosuppression in muddy conditions in both confined and semi-confined herds, as the concentration of lymphocytes and basophils is significantly reduced. This may be associated with animals submitted to cold, wet conditions and with more mud in the pens (Chen et al., 2017). In the present study, the effects of both

farm production system on the occurrence of uterine diseases were evaluated. The differences between the compost barn system and the semi-confined system did not significantly influence the analyses.

Buso et al. (2018) reported that the occurrence of retained placenta had no relation with the season that calving occurred. However, subclinical endometritis was more prevalent in cows that calved during the rainy

season, when uterine infection risk increases due to greater environmental contamination. Further, the authors concluded that retained placenta negatively affected reproductive performance of the evaluated cows by increasing days open the number of inseminations per conception, and the culling rate. Consistently, in the present study, cows with a perineal hygiene score of 2 or 3 presented a numerically lower conception rate (39.5%) than cows with a perineal hygiene score of 1 (48.9%). In addition, the average days open was increased (119.4 vs. 100.83

days) as a result of a higher number of artificial inseminations per conception (2.55 vs. 2.15 respectively) (Table 2). This unfavorable impact reduces reproduction reproductive efficiency; resulting in increased costs due to longer calving intervals; a decrease in the number of animals for replacement; a delay in genetic progress (Lima et al., 2010; Ribeiro et al., 2012); and higher involuntary culling and expenses related to animal nutrition, labor, and treatment, all of which affect the stayability of cows and the calving interval length (Giordano et al., 2011).

Table 2

Influence of the perineal hygiene score on the conception rate, the culling rate, days open, and the number of artificial inseminations per conception in crossbreed dairy cows in the Triângulo Mineiro region, 2020

Perineal score (n)	Conception rate	Culling rate	Days open (mean ± SE)	Number of artificial inseminations per conception (mean ± SE)
1 (50)	48.9%	6.0%	100.83 ± 9.9	2.15 ± 0.25
2 + 3 (43)	39.5%	11.6%	119.4 ± 11.0	2.55 ± 0.28

n - number; SE - standard error.

Campos et al. (2020) reported that the occurrence of postpartum diseases and clinical mastitis in a crossbreed dairy cows increased the number of artificial inseminations/conceptions. Dubuc et al. (2011) indicated that uterine diseases reduce the conception rate and increase embryo loss and days open. Uterine health during the postpartum period is also associated with the occurrence of uterine disorders. Rezende et al. (2020) observed the occurrence of at least one uterine disorder (22.6% for placenta retention, 14.3% for metritis, and 11.9% for clinical endometritis from 170 cows

among 588 animals) in postpartum cows presenting different uterus involution scores ($P < 0.001$). The most significant association was for scores of 2 and 3, where the uterus was still between the pelvic cavity and the abdominal cavity, and it was susceptible to contamination.

Rueda et al. (2015) demonstrated an association between restless animals with a tendency to respond to stressful situations by swinging their tails more frequently during management routine, which increases the risk of spreading feces and urine in the

perineal region. These behavioral responses could increase the artificial insemination practice time per animal and increase the risk of contamination.

According to Buso et al. (2018), crossbreed dairy cows, which present lower milk production levels than purebred animals, are less metabolically challenged during the postpartum period, leading to mildly compromised immune function. Thus, it is possible for these animals to recover spontaneously from uterine diseases, resulting in a smaller impact on reproductive efficiency (Campos et al., 2020). However, besides presenting a reduction in milk production, cows that present clinical health problems also often have milk disposal due to the treatment adopted, leading to relevant economic losses. These postpartum uterine disorders seem to affect the long-term physiology of the animals, compromising performance even after clinical recovery, which will impact the reproductive performance and farm profitability (Carvalho et al., 2019; Ribeiro et al., 2016).

Conclusion

At calving, crossbreed dairy cows that had a perineal region that is wet and/or covered with feces presented during postpartum a higher incidence of retained placenta, metritis, and clinical endometritis compared with cows that had a completely dry and clean perineal region. However, it was not possible to determine the effect of the perineal hygiene score on the reproductive efficiency.

Acknowledgements

We acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; research productivity grant to R. M. Santos). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

- Bellows, D. S., Ott, S. L., & Bellows, R. A. (2002). Review: cost of reproductive diseases and conditions in cattle. *Professional Animal Science*, 18(1), 26-32. doi: 10.15232/S1080-7446(15)31480-7
- Buso, R. R., Campos, C. C., Santos, T. R., Saut, J. P. E., & Santos, R. M. (2018). Retenção de placenta e endometrite subclínica: prevalência e relação com o desempenho reprodutivo de vacas leiteiras mestiças. *Pesquisa Veterinária Brasileira*, 38(1), 1-5. doi: 10.1590/1678-5150-PVB-4707
- Campos, C. C., Prado, F. L., Reis, J. P. J., Carneiro, L. C., Silva, P. R. B., Moraes, G. F., & Santos, R. M. (2020). Effects of clinical mastitis and puerperal diseases on reproductive efficiency of dairy cows. *Tropical Animal Health and Production*, 52, 3061-3068. doi: 10.1007/s11250-020-02326-2
- Carvalho, M. R., Peñagaricano, F., Santos, J. E. P., De Vries, T. J., & McBride, B. W. (2019). Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. *Journal Dairy Science*, 102(12), 11701-11717. doi: 10.3168/jds.2019-17025

- Chen, J. M., Stull, C. L., Ledgerwood, D. N., & Tucker, C. B. (2017). Muddy conditions reduce hygiene and lying time in dairy cattle and increase time spent on concrete. *Journal of Dairy Science*, *100*(3), 2090-2103. doi: 10.3168/jds.2016-11972
- Corrêa, M. N., Maffi, A. S., Riet-Correa, B., Cavazini, I. M., Antunes, M. M., Rabassa, V. R., & Brauner, C. C. (2014). Transtornos metabólicos no período de transição da vaca leiteira com ênfase nas enfermidades uterinas e da glândula mamária. In F. H. D. Gonzáles, A. C. R. Berreta, A. R. Guadagnin (Eds.), *A vaca leiteira do século 21: lições de metabolismo e nutrição* (pp. 127-151). Porto Alegre. <http://hdl.handle.net/10183/237269>
- Djuricic, D., Vince, S., Ablondi, M., Dobranic, T., & Samardzija, M. (2012). Intrauterine ozone treatment of retained fetal membrane in Simmental cows. *Animal Reproduction Science*, *134*(3-4), 119-124. doi: 10.1016/j.anireprosci.2012.08.023
- Dubuc, J., Duffield, T. F., Leslie, K. E., Walton, J. S., & Leblanc, S. J. (2011). Efeitos das doenças uterinas pós-parto na produção de leite abate de vacas leiteiras. *Journal Dairy Science*, *94*(3), 1339-1346. doi: 10.3168/jds.2010-3758
- Fernandes, C. A. C., Costa, D. S., & Viana, J. H. M. (2001). Impacto da retenção de placenta sobre a performance reprodutiva de vacas leiteiras. *Revista Brasileira de Reprodução Animal*, *25*(1), 26-30. <http://cbra.org.br/br/>
- Giordano, J. O., Fricke, P. M., Wiltbank, M. C., & Cabrera, V. E. (2011). An economic decision-making support system for selection of reproductive management programs on dairy farms. *Journal of Dairy Science*, *94*(12), 6216-6232. doi: 10.3168/jds.2011-4376
- Husnain, A., Arshad, U., Poindexter, M. B., Zimpel, R., Marinho, M. N., Perdomo, M. C., Fan, P., Jeong, K. C., Nelson, C. D., Sheldon, I. M., Bromfield, J. J., & Santos, J. E. P. (2023). Induced endometritis in early lactation compromises production and reproduction in dairy cows. *Journal of Dairy Science*, *106*(6), 4198-4213. doi: 10.3168/jds.2022-22846.
- Kronfeld, H., Kemper, N., & Hölzel, C. S. (2022). Vaginal and uterine microbiomes during puerperium in dairy cows. *Agriculture*, *12*(3), 41-18. doi: 10.3390/agriculture12030405
- Leblanc, S. J., Duffield, T. F., Leslie, K. E., Bateman, K. G., Keefe, G. P., Walton, J. S., & Johnson, W. H. (2002). Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *Journal Dairy Science*, *85*(9), 2223-2236. doi: 10.3168/jds.S0022-0302(02)74302-6
- Lima, F. S., De Vries, A., Risco, C. A., Santos, J. E. P., & Thatcher, W. W. (2010). Economic comparison of natural service and timed artificial insemination breeding programs in dairy cattle. *Journal of Dairy Science*, *93*(9), 4404-4413. doi: 10.3168/jds.2009-2789
- McDougall, S. (2001). Effect of intrauterine antibiotic treatment on reproductive performance of dairy cows following periparturient disease. *New Zealand Veterinary Journal*, *49*(4), 150-158. doi: 10.1080/00480169.2001.36223

- Miranda-CasoLuengo, R., Lu, J., Williams, E. J., Miranda-CasoLuengo, A. A., Carrington, S. D., Evans, A. C. O., & Meijer, W. G. (2019). Delayed differentiation of vaginal and uterine microbiomes in dairy cows developing postpartum endometritis. *PLoS One*, *14*(1), e0200974. doi: 10.1371/journal.pone.0200974
- National Research Council (2001). *Nutrient requirements of beef cattle* (8th ed.). The National Academy Press.
- Potter, T. J., Guitian, J., Fishwick, J., Gordon, P. J., & Sheldon, I. M. (2010). Risk factors for clinical endometritis in postpartum dairy cattle. *Theriogenology*, *74*(1), 127-134. doi: 10.1016/j.theriogenology.2010.01.023
- Ramos, O. P., Rezende, A. L., Alvarenga, P. B. de, Campos, C. C., Rezende, E. V. de, Silva, M. J. B., Carneiro, L. C., Moraes, G. F. de, Saut, J. P. E., & Santos, R. M. dos. (2022). Effect of retained placenta and clinical mastitis on reproduction parameters, immune response, and steroidogenic receptors gene expression in postpartum crossbred dairy cows. *Tropical Animal Health and Production*, *54*(3), 180. doi: 10.1007/s11250-022-03140-8
- Rezende, E. V., Campos, C. C., Moraes, G. F., & Santos, R. M. (2020). Factors related to uterine score and its influence on pregnancy per artificial insemination in crossbred dairy cows. *Livestock Science*, *241*, 1871-1413. doi: <https://doi.org/10.1016/j.livsci.2020.104231>
- Ribeiro, E. S., Galvão, K. N., Thatcher, W. W., & Santos, J. E. P. (2012). Economic aspects of applying reproductive technologies to dairy herds. *Animal Reproduction*, *9*(3), 370-387. <https://www.animal-reproduction.org/article/5b5a605af7783717068b46f4>
- Ribeiro, E. S., Gomes, G., Greco, L. F., Cerri, R. L. A., Vieira, A., Neto, Monetiro, P. L. J., Jr., Lima, F. S., Bisinotto, R. S., Thatcher, W. W., & Santos, J. E. P. (2016). Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. *Journal Dairy Science*, *99*(3), 2201-2220. doi: 10.3168/jds.2015-10337
- Rueda, P. M., Sant'anna, A. C., Valente, T. S., & Paranhos da Costa, M. J. R. (2015). Impact of temperament of Nellore cows on the quality of handling and pregnancy rates in fixed-time artificial insemination. *Livestock Science*, *177*, 189-195. doi: 10.1016/j.livsci.2015.04.021
- Schuenemann, G. M. (2010). Dairy calving management: effect of perineal hygiene scores on metritis. *Journal of Dairy Science*, *96*(4), 2671-2680. doi: 10.3168/jds.2012-5976
- Semambo, D. K., Ayliffe, T. R., Boyd, J. S., & Taylor, D. J. (1991). Early abortion in cattle induced by experimental intrauterine infection with pure cultures of actinomyces pyogenes. *Veterinary Records*, *129*(1), 12-16. doi: 10.1136/vr.129.1.12
- Sheldon, I. M. (2014). Genes and environmental factors that influence disease resistance to microbes in the female reproductive tract of dairy cattle. *Reproduction Fertility Development*, *27*(1), 72-81. doi: 10.1071/RD14305
- Sheldon, I. M., & Dobson, H. (2004). Postpartum uterine health in cattle. *Animal Reproduction Science*, *82*, 295-306. doi: 10.1016/j.anireprosci.2004.04.006

Sheldon, I. M., Cronin, J., Goetze, L., Donofrio, G., & Schuberth, H. J. (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biology of Reproduction*, *81*(6), 1025-1032. doi: 10.1095/biolreprod.109.077370

Sheldon, I. M., Lewis, G. S., Leblanc, S., & Gilbert, R. O. (2006). Defining postpartum uterine disease in dairy cattle. *Theriogenology*, *65*(8), 1516-1530. doi: 10.1016/j.theriogenology.2005.08.021

Walker, C. G., Meier, S., Hussein, H., Mcdougall, S., Burke, C. R., Roche, J. R., & Mitchell, M. D. (2015). Modulation of the immune system during postpartum uterine inflammation. *Physiological Genomics*, *47*(4), 89-101. doi: 10.1152/physiolgenomics.00098.2014

