

Uterine inflammation and metabolism: exploring postpartum events in primiparous and multiparous girolando dairy cows raised in the Amazon

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ABSTRACT

Despite its importance for the productive efficiency in Amazonian dairy systems, postpartum metabolism and uterine recovery in crossbred cows raised in this biome remain poorly understood. We compared the biological activity of the uterus and the metabolism between primiparous and multiparous crossbred dairy cows (*B. indicus* x *B. taurus*) in southwestern Amazonia. Cows were divided by parity order into primiparous (n = 8), and multiparous (n = 10) and were evaluated from parturition to 50 days in milk (DIM) for metabolic and reproductive variables. Uterine tissue was collected using the cytobrush technique to evaluate uterine inflammation and receptivity. Parity did not significantly affect energy metabolism, and multiparous cows had lower uterine inflammatory response associated with lower relative expression of *IL-6* and higher relative expression of *IL-10*. We conclude that parity has little effect on the puerperium of crossbred dairy cows, which were considered healthy by 50 DIM.

KEYWORDS: *Bos indicus*, fertility, metabolism, postpartum, uterus

Inflamação uterina e metabolismo: explorando eventos pós-parto em vacas leiteiras girolando primíparas e múltíparas criadas na Amazônia

RESUMO

Apesar de sua importância para a eficiência produtiva nos sistemas de produção leiteira da Amazônia, o metabolismo pós-parto e a recuperação uterina em vacas mestiças criadas nesse bioma ainda são pouco compreendidos. Comparamos a atividade biológica do útero e o metabolismo de vacas leiteiras mestiças primíparas e múltíparas (*B. indicus* x *B. taurus*). Dezoito vacas foram divididas por paridade em primíparas (n = 8) e múltíparas (n = 10) e foram avaliadas desde o parto até os 50 dias em lactação (DEL) quanto a variáveis metabólicas e reprodutivas. O tecido uterino foi coletado pela técnica cytobrush para avaliar a inflamação e receptividade uterina. A paridade não afetou significativamente o metabolismo energético e que vacas múltíparas apresentaram menor resposta inflamatória uterina associada à menor expressão relativa de *IL-6* e maior expressão relativa de *IL-10*. Concluímos que a paridade teve pouco efeito no puerpério de vacas leiteiras mestiças, que foram consideradas saudáveis aos 50 DEL.

PALAVRAS-CHAVE: *Bos indicus*, fertilidade, metabolismo, pós-parto, útero

Crossbred dairy cows (Holstein x Gir) are vital to the economy of developing countries (Leroy et al. 2015), especially where dairy herds are under tropical conditions, as in the Brazilian Amazon. Although there are several studies on the uterine health and energy balance of postpartum dairy cows (Leblanc

2012; Krause et al. 2014; Lietaer et al. 2022), few have assessed the interaction between metabolism, uterine repair dynamics, and gene expression associated with inflammation and uterine receptivity during postpartum in tropical conditions (Pfeifer et al. 2018, Andrade et al. 2021, 2024). Knowledge

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on postpartum uterine involution and health is important to define an adequate voluntary waiting period to provide more opportunities for cows to conceive and improve overall reproductive performance. Additionally, in intensive dairy production systems, typical of Brazil's subtropical regions, high-producing cows in their third and fourth lactations show lower reproductive efficiency compared to primiparous cows, mainly because higher milk production increases metabolic stress during early lactation and contribute to a longer postpartum anestrus (Inchaisri et al. 2010, Pfeifer et al. 2023). This reduction is linked to increased metabolic and reproductive demands. In contrast, under extensive tropical pasture-based systems, data on differences in reproductive efficiency related to parity remain scarce.

We hypothesized that multiparous crossbred dairy cows in the Amazon have more pronounced negative energy balance and longer postpartum anestrus than primiparous cows. We aimed to evaluate the relationship between parity and uterine health and metabolism of crossbred dairy cows in the postpartum period to understand the puerperium dynamics that may support reproductive management strategies to increase fertility.

The study was carried out on the experimental farm of the Brazilian Agricultural Research Corporation (Embrapa) (08°48'12"S, 63°50'56"W), in Porto Velho, Rondônia state, Brazil. The study was authorized by protocol # 03/2017 of the committee for ethics in animal experimentation (CEUA/Embrapa Rondônia).

We used 18 crossbred (*B. indicus* x *B. taurus*) dairy cows (8 primiparous and 10 multiparous; 5/8 Holstein x 3/8 Gir), grazing marandu grass (*Urochloa brizantha*), supplemented with corn and soybean concentrate (22% crude protein, 80%

total digestible nutrients). Cows were evaluated on the day of calving and at 10, 20, 30, 40, and 50 days in milk (DIM). We assessed body weight (BW) by individually weighing each cow, the body condition score (BCS) by using the scale from 1 to 5 with 0.25 increment units by Edmonson et al. (1989), and subcutaneous fat thickness (SFT) by using ultrasonography (Mindray M5; equipped with a 5 MHz linear transducer, Shenzhen, China) following Ayres et al. (2009). Milk production was evaluated at 10, 25, 40 e 55 DIM. The concentrations of β -hydroxybutyrate (β HB) and glucose were measured with a manual meter (TD - 4235[®]; Ketovet, MG, Brazil; Accu-Chek[®] Active, Roche, SP, Brazil, respectively), according to the manufacturer's instructions.

Cows underwent vaginoscopy at 5, 10, 15, 20, 25, 30, 40, and 50 DIM for assessing vaginal discharge, graded on a 0 to 3 scale, where positive purulent discharge (PVD+) was defined as ≥ 1 . Following PVD assessment on days 10, 20, 30, 40, and 50, cows had endometrial tissue collected for inflammatory cytological analysis and gene expression via the cytobrush technique (Pfeifer et al. 2018) using sanitary sheaths to avoid contamination by vaginal epithelial cells. Cows were considered healthy when the proportion of polymorphonuclear PolyMorphonuclear Neutrophils (PMN) in the uterus was $\leq 5\%$ and the PVD assessment was equal to 0 at 40 DIM.

Total RNA extraction, cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR) were performed to assess the relative abundance of mRNAs in the cytobrush samples from the endometrial samples (Pfeifer et al. 2018). All RNA samples were treated with deoxyribonuclease (DNase) prior to reverse transcription. Primer sequences used for qRT-PCR amplification of mRNA of the target genes are shown in Table 1.

Table 1. Primer sequences for quantitative real-time polymerase chain reaction (qRT-PCR) amplification of mRNA.

Gene name	Gene ID	Sequence forward	Sequence reverse	Sequence ID
Interleucin 1 beta	<i>IL-1β</i>	AGCATCTTTTCATTCATCTTTGAAG	CCTGTCATCTTCGAAACGTCCTCCGA	M37211
Interleucin 6	<i>IL-6</i>	GGGCTCCCATGATTGTGGTA	TTCCTGGGCATTCCCTCCTCTGGT	BC123577
Interleucin 8	<i>IL-8</i>	TGCTTTTTTGTTCGGTTTTTG AACAGGCACCTCGGGAATCCT	TAATCTTGCAACCTCACCCTGCTGGC	AF232704
Interleucin 10	<i>IL-10</i>	AGAACCACGGGCTGACATAGCTCACTGAAGAC TCTCTTCACCTT	TTCTGCCCTGCGAAACAAGAGCAA	U00799
Factor growth tumoral alpha	<i>TNFα</i>	TCTTCTCAAGCCTCAAGTAACAAGT CCATGAGGGCATGGCATACT	AGCCACGTTGTAGCCGACATCAACTC	NM_173966
Progesterone receptor	<i>PGR</i>	GCCGACGGTCTACCAGCCCTA	GTTATGCTGCTCTCCATTGCCCTT	NM_001205356.1
Estrogen receptor 1	<i>ESR1</i>	CAGGCACATGAGCAACAAG	TCCAGCAGCAGGTCGTAGAG	NM_001001443.1
Estrogen receptor 2	<i>ESR2</i>	TCACGTCAGGCAGCCAGTAAC	CACCAGGTTGCGCTCAGACCC	NM_174051.3
Patched 2	<i>PTCH2</i>	CATCCTGCTGCTGTACTT	ATCGCCAGGACCGACTACTAT	XM_005197904.1
Heparin binding EGF-like growth factor	<i>HB-EGF</i>	CATCCACGGAGAATGCAAATAC	CAGCAGACAGACGGATGATAG	NM_001144090.1
Glyceraldehyde-3-phosphate dehydrogenase	<i>GAPDH</i>	ACACTGAGGACCAGGTTG	TGGTCGTTGAGGGCAATG	NM_001034034.2
Actin β	<i>ACTB</i>	AGGCATCCTGACCCTCAAGTA	GCTCGTTGTAGAAGGTGTGGT	NM_173979.3

Parity was considered an explanatory variable (primiparous or multiparous). Response variables were evaluated using repeated measures ANOVA with the MIXED procedure to test the main day effects, parity, and their interactions using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). The proportion of healthy cows at 40 DIM was analyzed using the Chi-square test. The significance level was set at $P \leq 0.05$, and P values between 0.05 and 0.1 were considered a trend.

Of the 18 initial cows, one primiparous and three multiparous (22.2%) presented retained placenta (RP) and were excluded from the study. This incidence of RP needs to be investigated in further studies. All variables responded significantly to time (days), except β HB and SFT (Figure 1). No effect of parity was detected in BW, BCS, β HB, and SFT (Figure 1a-d).

No significant differences were observed in the proportion of PMN cells and PVD positivity between multiparous and

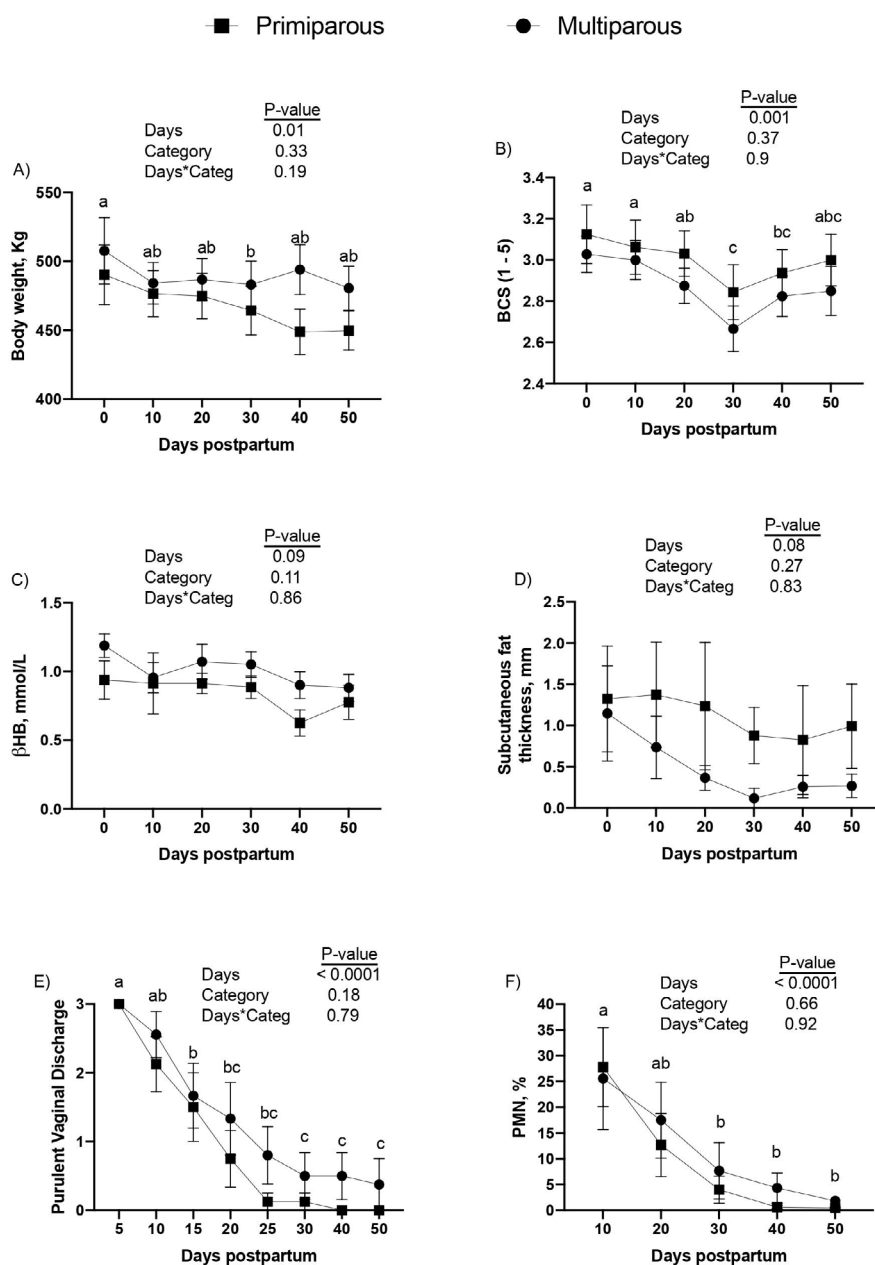


Figure 1. Evolution of body weight (A), body condition score (B), β -hidroxi butyrate (C), subcutaneous fat thickness (D), purulent vaginal discharge (E; grade 0-3), and proportion of PMN (F) from parturition to 50 DIM in primiparous ($n = 7$) and multiparous ($n = 7$) crossbred dairy cows in the southwestern Amazon. Statistical analysis considered the effects of parity, days from calving and the interaction between both variables. Squares and circles indicate the mean and bars indicate the standard errors. Different letters above the bars indicate statistical differences.

primiparous cows. However, the proportion of PMN cells and PVD positivity decreased significantly with time (Figure 1e-f). Twelve out of 14 cows (85.7%) were considered healthy by 40 DIM. Multiparous cows produced significantly more milk in the period than primiparous (Figure 2). Our lactation curve differed from studies with Holstein cows (Tekerli et al. 2000; Pfeifer et al. 2023), but the reduction in milk production after 30 DIM is consistent for Girolando cows raised in the Amazon (Lima et al. 2022).

Neither time nor parity or their interaction significantly affected the relative abundance of *TNFA*, *IL-1B*, *IL-8*, and *ESR1* mRNA, while the relative abundance of *IL6*, *HB-EGF*, *ESR2*, *PTCH2*, and *PGR* mRNA varied significantly with time (Figure 3). The relative abundance of *IL-6*, *PTCH2*, and *PGR* mRNA was significantly higher in primiparous than in multiparous cows, while the latter had significantly higher relative abundance of *IL-10* mRNA and tended to a higher relative abundance of *ESR2* mRNA ($P = 0.09$).

Our results showed that crossbred dairy cows efficiently recover uterine health after parturition, providing further evidence that Girolando cows raised under tropical conditions can be considered as open as soon as 40 DIM, when, according to uterine health data, post-parturition conception would be most propitious. The quick interval to recover uterine health after calving may be associated with the lower milk production compared to Holstein cows, and the non-intensive pasture-based milk production system in the Amazon. The sharp decrease in BCS by 30 DIM for all cows likely reflects the cumulative energy deficit resulting from increased milk production and limited dry matter intake during early lactation, with multiparous cows reaching a lower nadir than primiparous cows. A similar decrease in BCS after calving was observed in high-yielding Holstein cows (Pfeifer et al., 2023). Previously, Middleton et al. (2019) observed that cows that

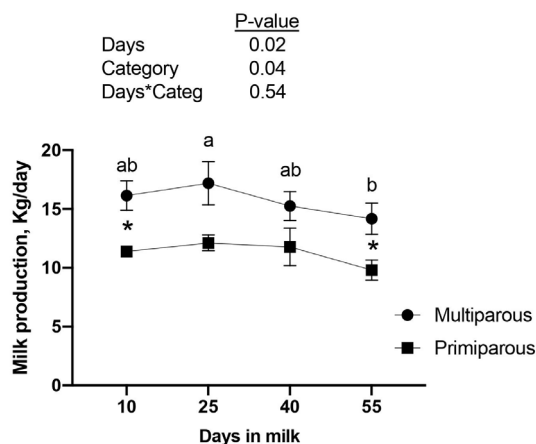


Figure 2. Milk production in primiparous and multiparous crossbred dairy cows after parturition in the southwestern Amazon. Squares and circles indicate the mean and bars indicate the standard errors. Different letters above the bar indicate statistical differences. Asterisks show statistical difference between the groups.

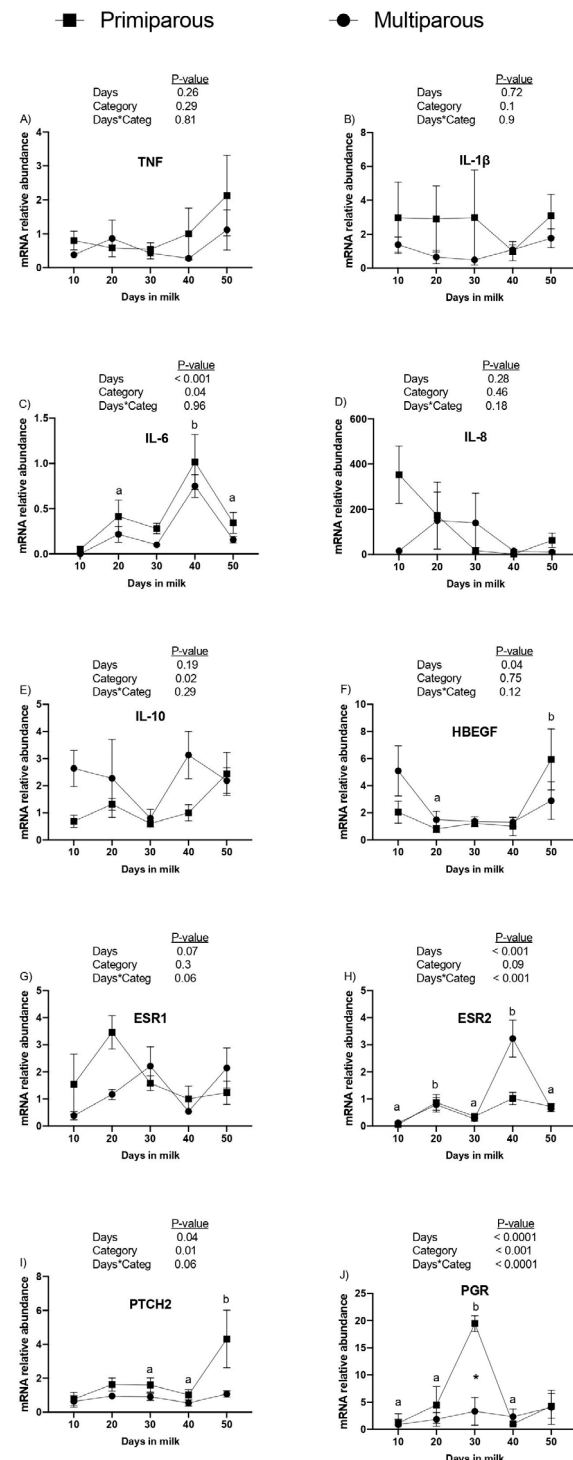


Figure 3. Relative abundance of pro-inflammatory cytokine transcripts for tumor necrosis factor alpha (A; *TNFA*), interleukin 1 beta (B; *IL-1β*), interleukin 6 (C; *IL-6*), interleukin 8 (D; *IL-8*), anti-inflammatory interleukin 10 (E; *IL-10*), heparin-binding EGF-like growth factor (F; *HB-EGF*), estrogen receptor 1 (G; *ESR1*), estrogen receptor 2 (H; *ESR2*), Patched 2a (I; *PTCH2*), regulation of cell proliferation progesterone receptor (J; *PGR*) mRNA in endometrial tissue of primiparous and multiparous crossbred dairy cows from 10 to 50 DIM in the southwestern Amazon. Squares and circles indicate the mean and bars indicate the standard deviation errors. Different letters above the bar indicate statistical differences.

lost less BCS after calving exhibited better fertility following the voluntary waiting period (VWP). Therefore, primiparous Girolando cows, which produce less milk, may show improved P/AI when enrolled in timed AI programs after the VWP, compared to multiparous cows. Further studies are needed to elucidate the differences in fertility between primiparous and multiparous Girolando dairy cows.

The elevated *IL-6* and reduced *IL-10* expression in primiparous cows suggest heightened uterine inflammation, marking pro- and anti-inflammatory states, respectively, while also showing favorable gene expression for endometrial repair and proliferation, exemplified by *PGR* and *PTCH2*. The increased *IL-6* and *ESR2* transcript levels by multiparous cows at 40 DIM suggest a modulated inflammatory response and enhanced estrogenic signaling. These changes may reflect parity-related adaptations affecting uterine recovery (Andrade et al. 2024). Additionally, *HB-EGF* transcript expression varied only temporally and is known to play a critical role in uterine tissue regeneration and embryo implantation (Takatsu and Acosta 2015). The study underscores temporal expression dynamics in postpartum bovine endometrium, with pro-inflammatory cytokines peaking early post-calving. In contrast, genes associated with repair and proliferation, like *PGR*, *ESR2*, and *PTCH2*, showed increased expression after 30 DIM.

We partially confirmed our hypothesis, as multiparous cows produced more milk than primiparous counterparts, but no disparities in energy status and uterine health between groups were observed. Gene expression analysis highlighted a superior pattern in genes associated with cell repair and proliferation (*PTCH2*, *PGR*) in primiparous cows, whereas multiparous cows exhibited a more suitable inflammation pattern (*IL-10*). Our results may be useful for designing new experiments to define an optimal VWP in crossbred dairy cows raised and managed in the Amazon.

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