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Exploring uterine inflammation in postpartum primiparous precocious and conventional and multiparous *Bos indicus* beef cows

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Abstract

This study aimed to compare the postpartum uterine dynamics of primiparous precocious (PP), primiparous conventional (PC) and multiparous conventional (MC) *Bos indicus* beef cows. For this purpose, PP ($n=8$), PC ($n=18$) and MC ($n=12$) cows were enrolled in this study. These cows were evaluated at 20 and 10 days prepartum and weekly from parturition to 42 days postpartum (DPP). During this period, body weight (BW), subcutaneous fat thickness (SFT) and serum concentrations of glucose, β-hydroxybutyrate, albumin and haptoglobin were measured. Proportion of polymorphonuclear (PMN) cells, and abundance of mRNA transcripts of genes involved in uterine inflammation and uterine health were evaluated. The PP cows had lower ($p<.05$) BW and SFT than that for PC and MC cows during the study period. The serum concentration of albumin after 35 DPP was lower ($p<.05$) in PP cows. The PP cows had the highest proportion of PMN on 28 and 35 DPP compared to PC and MC cows. The relative mRNA abundance of IL-1β and IL-8 increased after 21 DPP in PP cows compared to the other groups. The PC had the highest, MC had an intermediate, and PP cows had the lowest relative abundance of IL10 mRNA. Overall, these findings indicated that uterine inflammation was more pronounced in PP cows. Moreover, based on the proportion of PMN and abundance of transcripts associated with inflammation in the uterus, PP cows may require a longer period to recover their uterine health after calving.

KEYWORDS
Cattle, metabolism, ovulation, postpartum, uterus

1 | INTRODUCTION

Considering the need for intensive breeding in tropical beef production systems, timed artificial insemination (TAI) protocols are conventionally initiated as early as 30 days postpartum (Sa Filho et al., 2010; Vasconcelos et al., 2009). Such short calving-TAI may lead to postpartum TAI in cows with the inadequate reestablishment of uterine health for a new conception (Tomazi Filho et al., 2022).

In contrast to the intensive management used to breed postpartum *Bos indicus* beef cows, the same intensity cannot be easily achieved at the beginning of reproductive life in *Bos indicus* beef heifers. The age at puberty in *Bos indicus* beef heifers varies from 22 to 36 months (Nogueira, 2004). Aged heifers can significantly compromise the female reproductive longevity and profitability of the farm. Nonetheless, the beginning of the reproductive life of heifers at 14 months of age occurs in a few production systems where nutritional (Nepomuceno et al., 2017) and genetic (Eler et al., 2002; Ferraz Junior et al., 2018) strategies have been explored. Therefore, with adequate nutritional strategies from weaning to the first breeding season (Nepomuceno et al., 2017), *Bos indicus* heifers can be included in TAI programs as early as 14 months of age (Freitas et al., 2021). However, studies on uterine inflammation during the postpartum period have not yet been conducted.

Although, several studies have characterized NEB and postpartum uterine health in dairy cows (Galvao & Santos, 2010; Gilbert et al., 1993; Krause et al., 2014; Leblanc, 2012), few have assessed the association between postpartum metabolism and uterine health in *Bos indicus* beef cows raised under extensive tropical conditions (Andrade et al., 2021; Oliveira Filho et al., 2022; Pfeifer et al., 2018). To the best of our knowledge, studies comparing the postpartum uterine health of primiparous precocious (~24 months), primiparous conventional (>36 months), and multiparous conventional *Bos indicus* cows are scarce.

Based on these considerations, we hypothesized that younger *Bos indicus* beef cows are more susceptible to metabolic and a more pronounced and prolonged uterine inflammation following calving. Hence, in this study, we aimed to evaluate the relationships between animal categories and uterine inflammation, metabolism and resumption of ovarian activity in *Bos indicus* beef cows after calving. This study will help to understand puerperium dynamics that may further support postpartum reproductive management of *Bos indicus* beef cows raised in an extensive grazing system in the Amazon Biome.

2 | MATERIALS AND METHODS

2.1 | Animals

The Committee for Ethics in Animal Experimentation from Brazilian Agricultural Research Corporation (Embrapa) approved all the procedures performed in the experiment described in this manuscript (Protocol 03/2017).

This study was performed at the experimental farm of the Embrapa (Porto Velho, RO, Brazil, 08°48′12″ S, 63°50′56″ W). Thirty-eight Nelore (*Bos indicus*) cows were selected for this study. The animals were kept in *Brachiaria brizantha* pasture, with free access to water and mineral supplementation. Cows were grouped according to parturition order and age, as follows: Primiparous precocious (PP, $n = 8$), cows that calved once with 24 ± 2 months of age; Primiparous conventional (PC, $n = 18$), cows calved once with 36 ± 2 months of age, and Multiparous conventional (MC, $n = 12$), cows that calved ≥2 times. Cows that had retained placenta (RP) and/or other pathology during the experimental period were excluded from the study.

2.2 | Blood collection and metabolic assessments

Cows were evaluated on days −20 and −10, before calving and on days 7, 14, 21, 28, 35, and 42 postpartum to assess body weight (BW) and subcutaneous fat thickness (SFT). On the same days blood samples were collected to measure serum concentrations of glucose, β-hydroxybutyrate (βHB), albumin, and haptoglobin.

Ultrasonography (Mindray M5; equipped with a 5 MHz linear transducer) was used to measure the SFT. After immobilization of the animal, the evaluation site was cleaned with a brush with nylon bristles and vegetable oil was applied to increase the contact surface between the transducer and the animal's skin. Therefore, transducer was positioned linearly between coxal and iliac tuberosity and slightly moved until obtaining an adequate image that allowed the visualization of the upper limit of the *Biceps femoris*, as previously described by (Ayres et al., 2009).

Blood was collected by coccygeal venipuncture using vacuum tubes without anticoagulant (13 × 75 mm, 5 mL VacuTube®). The concentrations of β-hydroxybutyrate (βHB) and glucose were measured with a manual meter (TD-4235®; Ketovet; Accu-Chek® Active, Roche, respectively), according to the manufacturer's instructions. Commercial kit (LabTest®) was used to evaluate serum albumin concentrations using colorimetric spectrophotometry (Biospectro®, SP-220). Haptoglobin concentrations were measured according to the method that was previously described elsewhere (Jones & Mould, 1984).

2.3 | Gynaecological and reproductive assessments

Cows were examined by vaginoscopy to assess and characterize the presence of vaginal discharge on days 7, 14, 21, 28, 35 and 42 postpartum, as described elsewhere (Pfeifer et al., 2018). The presence of discharge was graded on a scale of 0 to 3 (0 = mucus, 1 = mucus with flecks of pus, 2 = ≥50% exudate purulent, 3 = haemorrhagic and/or purulent exudate), as adapted from others (Sheldon et al., 2006; Williams et al., 2005). Cows with positive purulent vaginal discharge (PVD+) were defined as cows having a vaginal discharge score of ≥1 (Pleticha et al., 2009). After PVD assessment, the cows were submitted to the collection of endometrial tissue for cytological analysis of

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

Gene name	Gene ID	Sequence forward	Sequence reverse	Function
Interleukin 1 Beta	*IL-1β*	CAAGGAGAGGAAAGAGACA	TGAGAAGTGCTGATGTACCA	Cytokine
Interleukin 6	*IL-6*	CCAGGAACGAAAGAGAGC	CAGAAGTCATCACCAGGAG	Cytokine
Interleucin 8	*IL-8*	CAAGAGCCAGAAGAAACCTGAC	AGTGTGGCCCACTCTCAATAAC	Cytokine
Interleucin 10	*IL-10*	AGAACCACGGGCCTGACAT	TTCTGCCCTGCGAAAACAAGAGCAA	Cytokine
		AGCTCACTGAAGACTCTCTTCACCTT		
Factor growth tumoral alpha	*TNF-α*	TCTTCTCAAGCCTCAAGTAACAAGT	AGCCCACGTTGTAGCCGACATCAACTC	Cytokine
		CCATGAGGGCATTGGCATAC		
Progesterone receptor	*PGR*	GCCGCAGGTCTACCAGCCCTA	GTTATGCTGTCCTTCCATTGCCCTT	Regulation of cell proliferation in the uterus
Oestrogen receptor 1	*ESR1*	CAGGCACATGAGCAACAAAG	TCCAGCAGCAGGTCGTAGAG	Regulation of cell proliferation in the uterus
Oestrogen receptor 2	*ESR2*	TCACGTCAGGCACGCCAGTAAC	CACCAGGTTGCGCTCAGACCC	Regulation of cell proliferation in the uterus
Patched 2[a]	*PTCH2*	CATCCTGCTGCTGTGTACTT	ATCGCCAGGACCAGTACTAT	Cell proliferation Embryogenesis
Heparin-binding EGF-like growth factor	*HB-EGF*	CATCCACGGAGAATGCAAATAC	CAGCAGACAGACGGATGATAG	Cell growth factor
Glyceraldehyde-3-Phosphate Dehydrogenase	*GAPDH*	ACACTGAGGACCAGGTTG	TGGTCGTTGAGGGCAATG	Reference genes
Actin β	B-Actin	AGGCATCCTGACCCTCAAGTA	GCTCGTTGTAGAAGGTGTGGT	Reference genes

the inflammatory condition of the uterus and gene expression using the cytobrush technique, as described elsewhere (Pfeifer et al., 2018).

Ultrasonic examination of the ovaries was performed on the same days of the cytobrush collections. The ovaries were evaluated to assess the presence of a corpus luteum (CL). Therefore, first postpartum ovulation was determined by the first postpartum detection of the CL on ultrasound assessments of the ovaries (Adapted from Mesquita et al., 2014).

2.4 | RNA isolation, cDNA synthesis and quantitative real-time polymerase chain reaction

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 as described elsewhere (Pfeifer et al., 2018). Primer sequences for qRT-PCR amplification of mRNA of the target genes used in this study are shown (Table 1). The changes in gene transcription were calculated by the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001) using the selected reference genes (βactin and GAPDH). Gene expression values of healthy multiparous cows (PVD = 0 and PMN ≤5%) at 35 DPP were used as a reference for all genes evaluated in this study because the uterus was within the expected standards for the bovine female have adequate fertility. The gene expression value for each gene from the reference samples was expressed as 1.0, and all other samples were calculated in relation to this value and then averaged. Then, all other values of the genes evaluated in the different DPP for multiparous and primiparous cows were divided by the mean value of the relative abundance of the reference value. In this way, it was possible to produce the fold change of the genes of interest compared to the reference value (Masternak et al., 2005).

2.5 | Statistical analyses

All statistical analyses were performed using SAS 9.0 software (SAS Institute Inc.). Variables for repeated measures (BW, βHB, glucose, BCS, PMN, vaginal discharge, IAR, SFT and relative abundances of mRNA) were evaluated using an analysis of variance (ANOVA) with the MIXED procedure to test the main day effects, category and their interactions. When discernible distinctions among categories on a particular day were identified, they have been meticulously delineated within the accompanying figures. The day of first ovulation was analysed using a one-way ANOVA. In all tests, the significance level was set at $p < .05$, and p values between .05 and .1 were considered as a trend.

3 | RESULTS

Of the 38 Nelore cows initially enrolled in the study, seven (one PP, three PC and three MC) retained their placenta after calving. Therefore, these cows were excluded from this study.

The data on energy metabolism are presented in Figure 1. The effect of time (days) was observed for all variables ($p < .05$) except for βHB. Precocious cows had a lower ($p < .05$) BW, SFT and βHB than that of PC and MC cows during the study period (Figure 1a,b,d). No differences in serum glucose ($p = .27$; Figure 1c), albumin ($p = .42$; Figure 1e) or haptoglobin ($p = .07$; Figure 1f) concentrations were observed among the groups. However, the serum albumin concentrations on 35 and 42 DPP were lower ($p < .05$) in the PP group than that in the PC and MC groups.

The proportions of uterine PMN cells and PVD during PPP are shown in Figure 2. For PVD, there were effects of days, category and days × category interaction (Figure 2a). The PP cows had higher ($p = .03$) PVD than that for PC and MC cows on days 28, 35 and 42 postpartum. The proportion of PMN changed according to the category and day × group interactions. On 7 DPP, PP had a lower PMN than that for PC and MC cows. In contrast, PP had a higher proportion of PMN on days 28 and 35 DPP than that for PC and MC cows (Figure 2b).

Data on the relative abundance of cytokine transcripts for *IL-1β*, *IL-6*, *IL-8*, *TNF-α* and *IL-10* mRNA in endometrial tissues of postpartum cows from 7 to 42 DPP are shown (Figure 3a–e). A categorical effect ($p < .05$) was observed in the abundance of *IL-1β* and *IL-8* mRNA (Figure 3b,c). The relative mRNA abundance of these genes increased after 21DPP in PP cows compared to PC and MC cows. Moreover, a day × category effect was observed for *IL-1β*, *IL-8* and *TNF-α* mRNA relative abundance. In PP cows, the relative abundance of these genes increased as the interval from calving increased (Figure 3b–d). Category affected ($p = .04$) the relative mRNA abundance of *IL-10*. Primiparous conventional cows had the highest, MC had an intermediate, and PP had the lowest relative abundance of *IL-10*.

Data on the relative abundances of *HB-EGF*, *PGR*, *PTCH2*, *ESR1* and *ESR2* mRNA are shown (Figure 3f–j). A day × category interaction effect was observed for all gene transcripts except for *ESR2*. The effect of the day on the relative abundance of *PGR*, *PTCH2* and *ESR1* mRNA (Figure 3g–i). A category effect was observed for the *PTCH2* transcripts (Figure 3h).

No effect of category was observed in the calving-first ovulation interval ($p = .25$). Only one PP cow ovulated during the experimental period (42 DPP). Two PC cows ovulated (28 ± 7.0 DPP) and four MC cows ovulated (33.2 ± 1.7 DPP) throughout the experimental period.

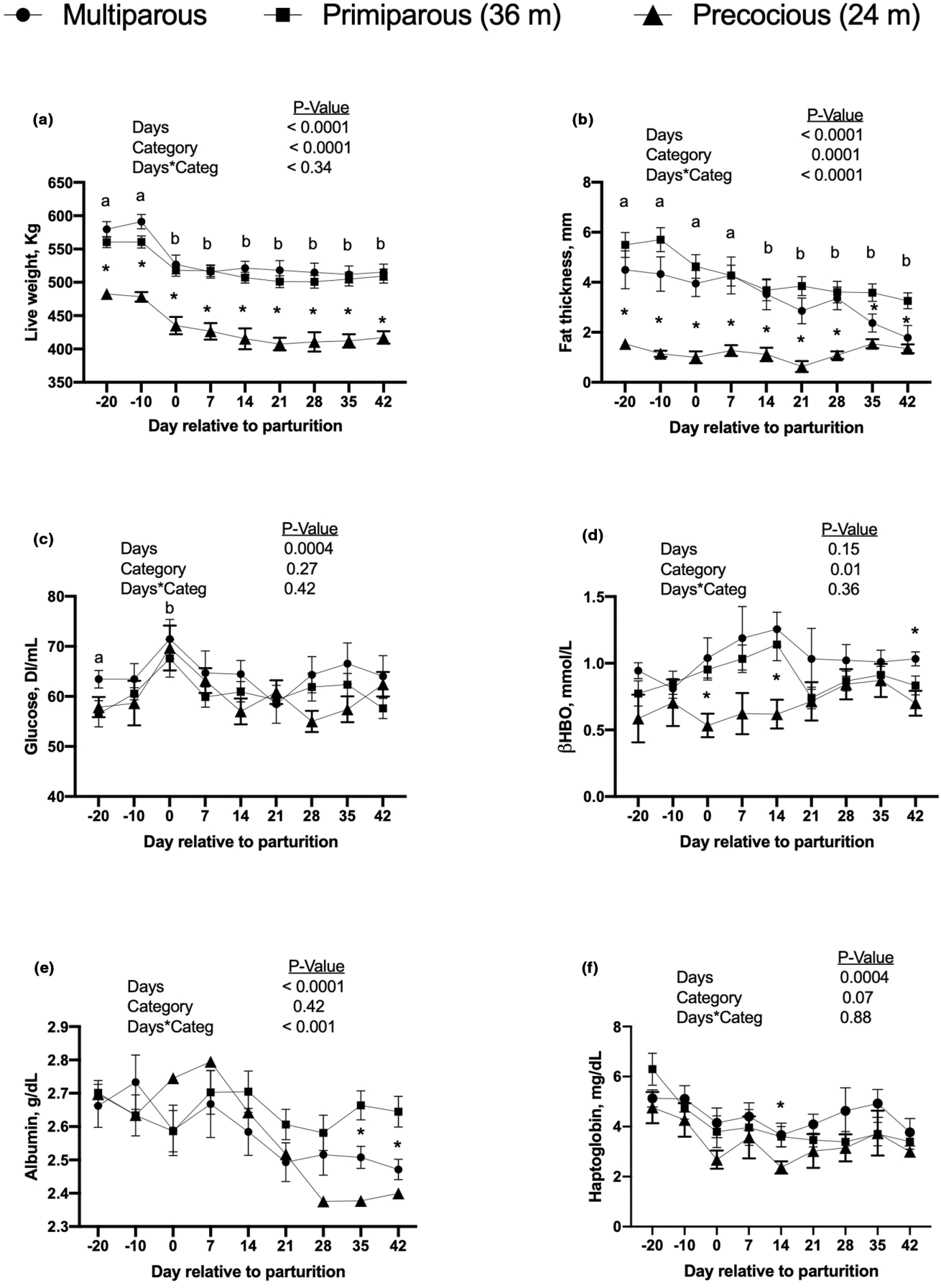
4 | DISCUSSION

In the present study, we compared the energy metabolism, uterine health and resumption of ovarian activity among PP, PC and MC *Bos indicus* beef cows from 20 days before to 42 days after parturition. The hypothesis of this study was partially confirmed. The PP cows had lower BW, SFT and βHB concentrations than that of PC and MC cows. Moreover, the lower serum concentration of albumin on 35 and 42 DPP in PP cows than that in PC and MC cows indicated that younger cows experienced higher inflammation patterns in the late postpartum period. Similarly, from an uterine perspective, PP cows had greater PVD and a greater proportion of PMN than that for PC and MC cows after 21 DPP. The abundance of transcripts of some genes associated with inflammation in the uterus (*IL-1β* and *IL-8*) increased as the interval from calving increased in the PP cows in contrast to that in older cows (PC and MC). To the best of our knowledge, this is the first study to characterize the relationship between uterine inflammation, metabolism and resumption of ovarian cyclicity and parity order in *Bos indicus* beef cows raised under tropical conditions. Moreover, no previous studies have characterized the postpartum dynamics of uterine inflammation in PP Nelore cows.

It may be speculated that PP cows are more susceptible to metabolic disorders following calving because of the increased metabolic demands for growth and development. Indeed, in the present study, PP cows had a lower BW and SFT than that for older cows. Nonetheless, they also had lower serum concentrations of βHB, indicating that they lost less fat tissue than older cows, as observed by comparing the loss of SFT between categories. Kasimanickam et al. (2013) proposed that the loss of body condition and resulting postpartum metabolic and endocrine changes prolong the uterine inflammatory process. In the present study, the PP cows lost 0.9 mm SFT, and the PC and MC cows lost >2 mm SFT during the experimental period. This result demonstrated that the greater uterine inflammation observed in PP cows was probably not associated with more pronounced NEB in these cows. However, further studies are needed to determine the cause of high inflammation patterns in this category.

The PP cows had greater PVD and a greater proportion of PMN cells in the uterus after 28 DPP than PC and MC cows. Contrary to the observations in PP cows, PVD and PMN decreased over time after calving in older PC and MC cows. Similarly, several studies have observed that uterine inflammation decreases over time after calving in dairy (Prunner et al., 2014; Tanai et al., 2020) and beef cows (Andrade et al., 2021; Pfeifer et al., 2018; Santos et al., 2009). Multiparous cows had only 0.6% PMN cells in their uterus on 35 DPP. A previous study indicated a cutoff of 35 DPP as a voluntary waiting period to include multiparous *Bos indicus* beef cows in E2-P4 based TAI protocols (Pfeifer et al., 2018). However, according to the

FIGURE 1 Measurements of body weight (a), subcutaneous fat thickness (b), internal angle of the rump (c) and serum glucose concentration (c), βHB (d), albumin (e) and haptoglobin (f) in precocious ($n = 7$), primiparous ($n = 15$) and multiparous ($n = 9$) beef cows from −20 to 42 days postpartum. Statistical analysis was performed for the pre and postpartum periods for the effects of category, days from calving and the interaction between category and days from calving. *Differences between categories on a given day are indicated (*). Panel a, From Day −20 to 42 (MC × PC, $p > .3$; MC × PP, $p < .001$; PC × PP, $p < .01$). Panel b, From Day −20 to 28 (MC × PC, $p > .2$; MC × PP, $p < .01$; PC × PP, $p < .001$), Days 35 and 42 (MC × PC, $p > .05$; MC × PP, $p > .05$; PC × PP, $p < .001$). Panel d, Days 0 and 14 (MC × PC, $p > .7$; MC × PP, $p < .05$; PC × PP, $p < .05$), Day 42 (MC × PC, $p = .08$; MC × PP, $p = .03$; PC × PP, $p = .51$). Panel e, Days 35 and 42 (MC × PC, $p < .05$; MC × PP, $p > .05$; PC × PP, $p < .02$). Panel f, Day 14 (MC × PC, $p = .99$; MC × PP, $p = .07$; PC × PP, $p = .04$). [ab]Different letters indicate effect of day.



present data for PP and PC beef cows, this voluntary waiting period should perhaps be reconsidered because the mean proportion of PMN cells at 35 DPP in these categories was 37.6% and 6%, respectively. Further field studies with larger numbers of animals should be performed to establish the relationship between calving-TAI interval and fertility in primiparous Nelore cows raised in extensive production systems.

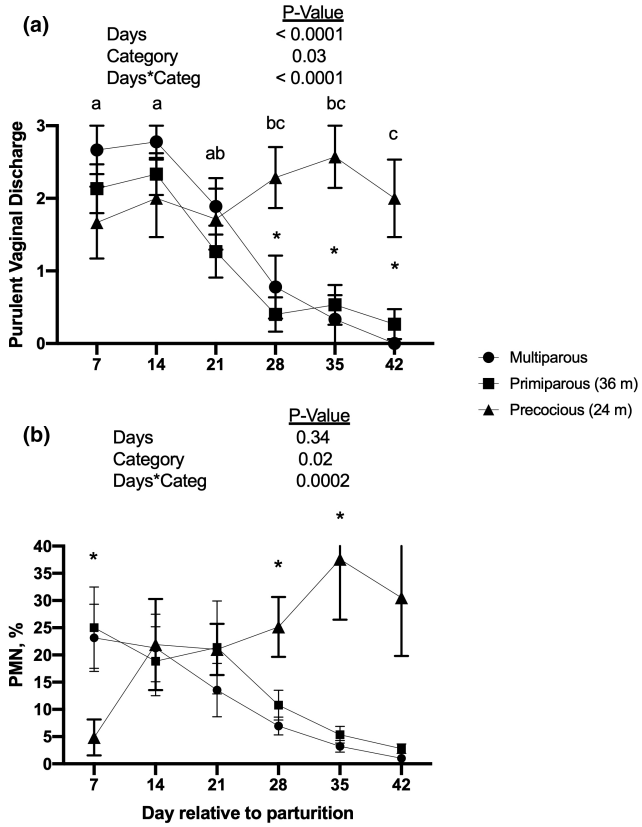
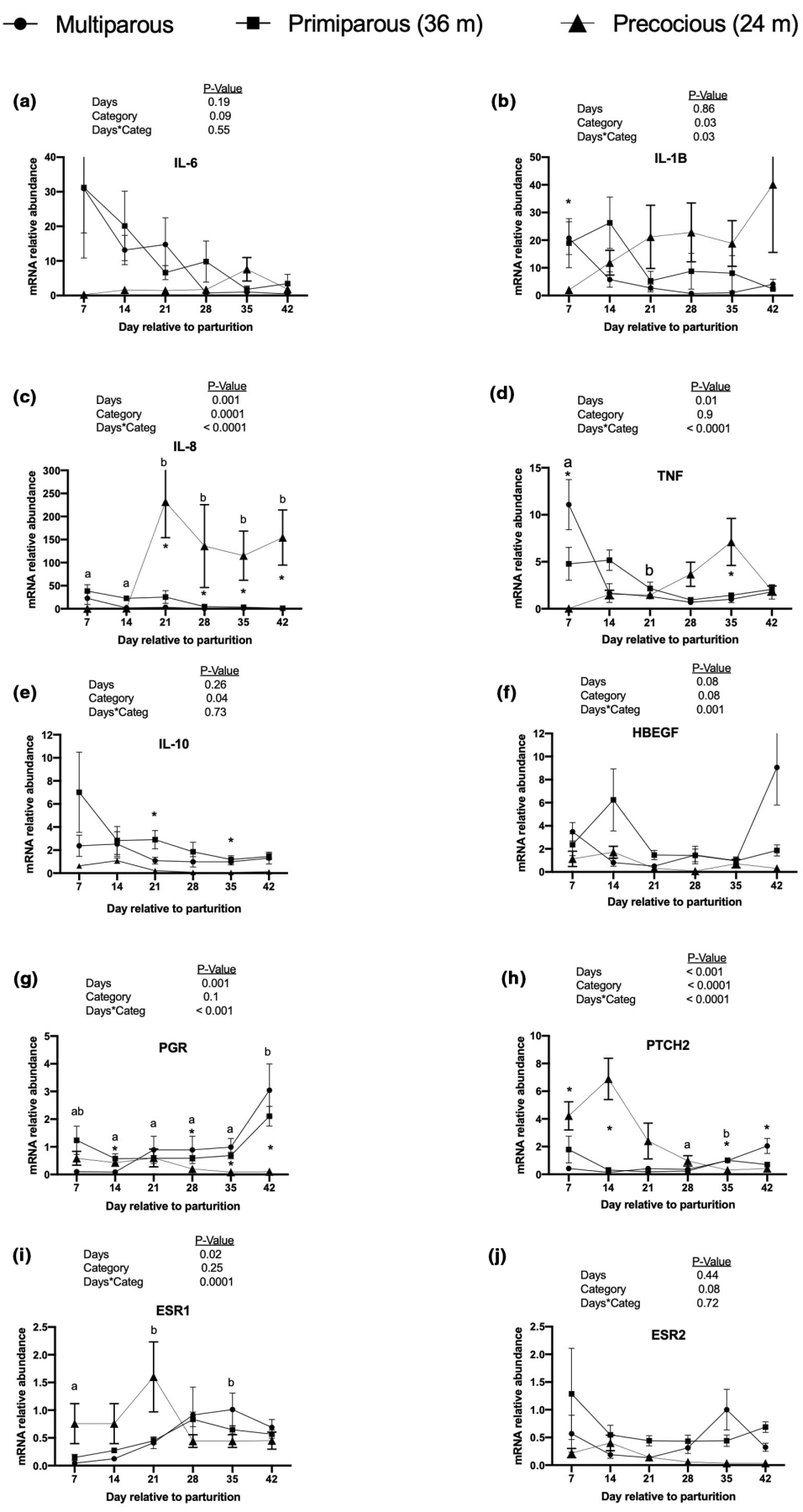


FIGURE 2 Purulent vaginal discharge (a; grade 0–3) and proportion of PMN (b; %) in precocious, primiparous and multiparous beef cows (mean ± SEM) from 7 to 42 days postpartum. Statistical analysis was performed for the pre and postpartum periods for the effects of category, days from calving and the interaction between category and days from calving. *Differences between categories on a given day are indicated (*). Panel a, From Day 28 to 42 (MC×PC, $p > .4$; MC×PP, $p < .05$; PC×PP, $p < .05$). Panel b, Day 7 (MC×PC, $p = .9$; MC×PP, $p = .04$; PC×PP, $p = .07$), Day 28 (MC×PC, $p = .4$; MC×PP, $p = .03$; PC×PP, $p = .1$) and Day 35 (MC×PC, $p = .5$; MC×PP, $p = .04$; PC×PP, $p = .05$). [ab]Different letters indicate the effect of day.

Studies have shown that ovarian steroids affect the immune response against uterine infections (Lewis, 1997, 2004). Therefore, the resumption of ovarian cyclicity and consequent increase in estradiol concentrations allow the uterine immune mechanism to eliminate pathogens early; thereby, decreasing the proportion of PMN cells in the uterus (Subandrio et al., 2000). The proportion of MC cows that ovulated during the experimental period tended to be greater than that of PC cows. Only one (14.3%) PP cow and two (13.3%) PC cows ovulated during the experimental period. The low proportion of primiparous cows that ovulated during the experimental period may be associated with the high proportion of PMN in the uterus after 35 DPP. Surprisingly, peaks in PMN and PVD in PP cows occurred only on day 35.

The expression of endometrial *IL-1β* and *IL-8* increased in PP cows, concomitantly with an increase in the proportion of PMN. This is the first study to characterize cytokines gene expression in PP cows. We previously characterized the gene expression patterns of cytokines in multiparous postpartum *Bos indicus* beef cows (Pfeifer et al., 2018). In that study, the endometrial gene expression of *IL-1β* and *IL-8* decreased as the postpartum period progressed. In contrast, in the present study the expression of endometrial *IL-1β* and *IL-8* increased as postpartum days increased in PP cows. Non-lactating Holstein cows that received an intrauterine infusion of endometrial pathogenic bacteria (*Escherichia coli* and *Trueperella pyogenes*) demonstrated an increased expression of endometrial inflammatory mediators and mucopurulent discharge in the vagina (Dickson et al., 2020). The authors observed that the expression of *IL-1β*, *IL-6* and *TNF* decreased as the number of days relative to infusion increased. In the present study, we did not use the intrauterine infusion to cause inflammation; however, similar to that observed in that study, the expression of *IL-6*, *IL-1β*, *IL-8* and *TNF* decreased as DPP increased in PC and MC cows. These findings clearly demonstrate that the intensity of the inflammatory response was associated with a high expression of proinflammatory cytokines, which was highly associated with a greater proportion of PMN cells and PVD. In this regard, the association between cytokine expression and the proportion of PMN and PVD in the uterus occurred earlier in PC and MC cows and later in PP cows after calving. Accordingly, we observed the lowest expression of the *IL-10* gene, a cytokine associated with the resolution of inflammation, in PP cows compared to PC and MC

FIGURE 3 Relative abundance of transcripts for interleukin 6 (a; *IL-6*), interleukin 1 beta (b; *IL-1β*), interleukin 8 (c; *IL-8*), tumour necrosis factor alpha (d; *TNF-α*), anti-inflammatory interleukin 10 (e; *IL-10*), heparin-binding EGF-like growth factor (f; HB-EGF), progesterone receptor (g; *PGR*), Patched 2a (h; *PTCH2*), oestrogen receptor 1 (i; *ESR1*) and oestrogen receptor 2 (j; *ESR2*) mRNA in endometrial tissues in precocious, primiparous and multiparous cows from 7 to 42 days postpartum. Statistical analysis tested the effects of category, days from calving and category*days. *Differences between categories on a given day are indicated (*). Panel b, Day 7 (MC×PC, $p = .9$; MC×PP, $p = .04$; PC×PP, $p = .2$). Panel c, from Day 21 to 42 (MC×PC, $p > .6$; MC×PP, $p < .001$; PC×PP, $p < .001$). Panel d, Day 7 (MC×PC, $p < .001$; MC×PP, $p < .001$; PC×PP, $p < .01$) and Day 35 (MC×PC, $p = .9$; MC×PP, $p < .02$; PC×PP, $p < .01$). Panel e, Day 21 (MC×PC, $p = .1$; MC×PP, $p = .04$; PC×PP, $p = .01$) and Day 35 (MC×PC, $p = .8$; MC×PP, $p = .03$; PC×PP, $p = .02$). Panel g, Day 7 (MC×PC, $p = .03$; MC×PP, $p = .02$; PC×PP, $p = .7$), Day 28 (MC×PC, $p = .82$; MC×PP, $p = .4$; PC×PP, $p = .01$) and Days 35 and 42 (MC×PC, $p > .6$; MC×PP, $p < .05$; PC×PP, $p < .001$). Panel h, Days 7 and 14 (MC×PC, $p > .3$; MC×PP, $p < .05$; PC×PP, $p < .05$), Day 35, (MC×PC, $p = .9$; MC×PP, $p = .02$; PC×PP, $p < .01$) and Day 42 (MC×PC, $p = .09$; MC×PP, $p = .04$; PC×PP, $p = .16$). [ab]Different letters indicate effect of day.



cows. The lowest abundance of *IL-10* transcripts was concomitantly observed with the greatest proportion of PMN in the uteri of these cows.

The expression of genes associated with endometrial repairs and proliferative activity, such as *HB-EGF*, *PGR*, *ESR1* and *ESR2*, did not differ between categories. However, endometrial *PTCH2* expression was higher in PP cows than that in PC and MC cows on 7 and 14 DPP. Interestingly, PP cows presented the lowest expression of *PTCH2* on 35 DPP, when a greater proportion of PMN cells was observed in the uterus. Accordingly, PP cows showed the lowest expression of *PTCH2*, *PGR* and *ESR2* on 35 DPP. In contrast, Sa Filho et al. (2017) observed that beef cows treated with estradiol cypionate showed increased expression of the proliferation-related candidate gene, *PTCH2*. Several studies have reported that gene expression in the endometrial tissue of cattle changes temporally depending on the phase of the oestrous cycle and is mainly orchestrated by the concentrations of E2, P4 and their receptors (Forde et al., 2009; Mesquita et al., 2014, 2015; Okumu et al., 2010; Sa Filho et al., 2017). In this regard, in the present study, we observed an increase in the expression of endometrial *PGR* after ovulation in MC cows, 33.2 ± 1.7 DPP. Accordingly, Foley et al. (2012) observed the enrichment of differentially expressed genes associated with tissue repair and proliferative activity at 30 DPP was observed by (Foley et al., 2012). An increase in E2 serum concentration has been associated with uterine modulation (Mesquita et al., 2014; Sa Filho et al., 2017) and the induction of P4 receptor synthesis in the endometrium (Lamming & Mann, 1995; Robinson et al., 2001). To the best of our knowledge, this is the first study to compare postpartum endometrial proliferative activity in MC, PC and PP beef cows. Evaluation of the expression patterns of these genes revealed important information regarding the repair process in the postpartum uterine mucosa, which may help understand the underlying molecular processes and define reproductive management strategies for postpartum *Bos indicus* beef cows.

5 | CONCLUSION

The results of this study show that the transcripts of inflammatory cytokines and genes related to endometrial proliferative repair and activity were differentially expressed during the postpartum period and among the categories. Proinflammatory *IL-1β* and *TNF* cytokine expression increased in the first weeks after parturition in PC and MC cows. In contrast, an increase in proinflammatory *IL-1β* and *IL-8* cytokine endometrial expression was observed as the DPP increased in PP cows. Overall, PP cows had the lowest abundance of transcripts of genes associated with uterine health (*PTCH2*, *ESR2* and *IL-10*) during the study period. In summary, our findings underscore the heightened postpartum uterine inflammation observed in PP cows, suggesting the need for an extended postpartum period to restore uterine health.

AUTHOR CONTRIBUTIONS

Jéssica de Souza Andrade and Elizângela Mírian Moreira contributed in investigation and writing. Vanessa Lemos de Souza, Ingrid Pedraça Barbosa, George Moreira Silva, Leonardo Silva Gomes, Samira Alves de Souza Silva, Gabrielly Cristina Santos Noleto and Renata Reis da Silva investigated and did the laboratory analyses; Uriel Secco Londero and Márcio Nunes Correa provided the methodology, resources, data curation and writing. Luiz Francisco Machado Pfeifer contributed in conceptualization, methodology, supervision, project administration, review and editing.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author.

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