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Effect of assisted calving on uterine puerperal disorders occurrence, and on reproductive efficiency in crossbred dairy cows

Efeito do parto assistido na ocorrência de desordens uterinas puerperais e na eficiência reprodutiva de vacas leiteiras mestiças

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Abstract
The aim of this study was to evaluate the effect of the type of calving (normal or assisted) on the occurrence of puerperal uterine disorders and on the number of artificial inseminations (AI) per conception and pregnancy rate at 150 days postpartum (PPD). Cows were observed during parturition and the type of calving was classified as normal or assisted. Normal calving required no human interference, while assisted calving required a calf removal aid via vaginal access. There were 825 births, 7 stillbirths (0.85%) and 17 twins (2.06%). We analyzed 801 single births, from which 766 (95.63%) were normal and 35 (4.36%) assisted. Were evaluated the uterine disorders occurrence such as retained placenta combined with metritis (PR/ME) and clinical endometritis (CE). The overall occurrences were 10.24% (82/801) of RP/ME, 12.86% for CE and 5.12% for RP/ME and CE. Most of normal calving cows had healthy puerperium (73.89%), whereas a majority of assisted calving cows had uterine disorders (74.29%). The number of AI per conception was similar for cows that had either normal or assisted calving (2.39±0.08 and 3.00±0.43, P = 0.16). There was no evidence of negative influence of calving type on the 150 PPD pregnancy rate of lactating crossbreed dairy cows (P = 0.44). Healthy cows had higher 150 PPD pregnancy rate than cows affected by puerperium uterine disorders (51.65 vs. 42.92%). Normal calving crossbred dairy cows had a healthier puerperium, compared to cows with assisted calving which were more susceptible to puerperium uterine disorders. And cows with a health puerperium have a higher pregnancy rate at 150 days postpartum.
Keywords: calve; metritis; puerperium; pregnancy rate.

Resumo
Objetivou-se avaliar em vacas leiteiras mestiças que pariram um bezerro vivo, o efeito do tipo de parto (normal ou assistido) na ocorrência de desordens uterinas puerperais e no número de inseminações artificiais (IA) por concepção e taxa de prenhez 150 dias pós-parto (DPP). As vacas foram monitoradas durante o parto e o tipo de parto foi classificado como normal ou assistido. O parto normal não precisou de interferência humana, enquanto o parto assistido precisou de auxílio para retirada do bezerro por via vaginal. Foram registrados 825 partos, sendo 7 natimortos (0,85%) e 17 gemelares (2,06%). Foram analisados 801 partos simples, dos quais 766 (95,63%) foram normais e 35 (4,36%) foram assistidos. Foram avaliadas a ocorrência de desordens uterinas como a retenção de placenta associada com metrite (RP/ME) e endometrite clínica (EC). A ocorrência das doenças foi de 10,24% (82/801) para RP/ME, 12,86% para EC e 5.12% para RP/ME com CE. A maioria das vacas com parto normal tiveram puerpério saudável (73,89%), enquanto a maioria das vacas com parto assistido apresentaram desordens uterinas (74,29%). O número de IA por concepção foi semelhante nas vacas que tiveram parto normal ou assistido (2,39±0,08 e 3,00±0,43, P = 0,16). Não houve evidência de influência negativa do tipo de parto na taxa de prenhez 150 DPP de vacas leiteiras mestiças em lactação (P = 0,44). Vacas saudáveis apresentaram maior taxa de prenhez 150 DPP do que vacas afetadas por desordens uterinas no puerpério (51,65 vs. 42,92%). Vacas leiteiras mestiças com parto normal tiveram um puerpério saudável, comparadas às que tiveram parto assistido, por sua vez foram mais susceptíveis as desordens uterinas no puerpério. E vacas com puerpério saudável resultaram em maiores taxas de prenhez aos 150 dias pós parto.
Palavras-chave: bezerro; metrite; puerpério; taxa de prenhez.

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1. Introduction

Dystocia is defined as a difficult birth resulting in prolonged calving or assisted removal of the calf at birth [1]. Assisted calving is defined as a birth in which assistance is required (e.g., correction of wrong positions). Minimum intervention is necessary in this scenario, whereas in dystocia births auxiliary traction devices are often needed, and may take considerable time and even lead to fetal death [1]. Occasional assistance during calving is important to ensure cow and calf survival. Early intervention has the potential to prevent stillbirths [2,3] while unnecessary or premature intervention can also cause injuries in the birth canal due to the lack of proper soft tissue dilation [1]. Although the prevalence of dystocia may appear to be low (between 4.1 and 13.7%), calving assistance rates are high, varying between 10 and >50% [1,4].

Dystocia increases the incidence of stillbirths [5,6] and calf mortality within 30 days post-calving [6,4]. The occurrence of dystocia negatively affects the productive and reproductive performance of dairy herds, as dystocia is related to a decrease in milk yield [7] and conception rate followed by an increase in days open and number of artificial inseminations (AI) per conception [8]. In addition, dystocia increases the likelihood of trauma in the reproductive tract of the dam, which results in uterine disorders [9].

Ascending bacterial contamination commonly occurs during and after calving and affects around 90% of cows at calving [10]. During uterine involution, bacterial content is eliminated from the uterus and the infection itself will only become established if pathogenic bacteria persist in the lumen [11]. The establishment of uterine disorders also depends on factors such as uterine damage, environment bacterial challenge, and the cow's nutritional and immunological status.

The high prevalence of uterine postpartum disorders is a challenge in terms of optimizing the reproductive efficiency of dairy herds. These disorders are directly related to a delay ovarian cyclicity, reduction in conception rates, increase the interval from calving to conception, pregnancy loss rates and the risk of culling for infertility [12-13]. Several predisposing factors of uterine infection, such as twin birth, calving assistance, dystocia, stillbirth, retained placenta (RP), abortion, metabolic disorders such as hypocalcemia, and a displaced abomasum have been reported [11,12].

We hypothesized that crossbred lactating dairy cows that had a single live calf without assistance would have a healthy postpartum period and better future reproductive performance than cows that need calving assistance. Thus, the aim was to evaluate in crossbred dairy cows that delivered a single living calve, the effect of type of parturition (normal or assisted) on puerperal uterine disorders occurrence, and on number of artificial inseminations (AI) per conception and pregnancy rate at 150 days postpartum (DPP).

2. Material and methods

2.1 Animals, environment, nutritional and sanitary management

This study was conducted on a commercial dairy farm, in Lagoa Santa in the North region of Minas Gerais, Brazil. Data was collected from May 2018 to December 2019. The herd was composed of crossbred dairy cows (Holstein x Gyr), with an average of 670 lactating cows mechanically milked three times per day (25.0 kg of milk/cow/day). The region's climate is considered tropical dry with average annual precipitation of 0.07 mm and THI ranging from 69 to 72. The average annual temperature of the region was 24 °C and average of humidity within 50 to 60%.

Throughout the year, lactating dairy cows were confined in loose housing, and received a total mixed ration (TMR) composed of corn silage, cotton seed, concentrate and minerals. All cows had *ad libitum* access to water and all diets were balanced according to each cow's productivity and formulated in accordance with the National Research Council's recommendations (NRC, 2001).

The annual health program of the farm, included vaccines for foot-and-mouth disease, brucellosis, bovine viral diarrhea virus (BVD), infectious bovine rhinotracheitis (IBR), leptospirosis, and clostridial diseases. The adult animals were also dewormed twice a year, and the drugs used were alternated. Cows were treated with bovine somatotropin (bST – Lactotropin®, Agener União, Brazil) every 14 days, starting from 60 DPP until day 190 of pregnancy. The use of bST is not prohibited in Brazil. The environment in which the cows were kept had a temperature and humidity controlling system in the waiting room of the milking parlor to keep the cows thermally comfortable. Cows were cooled for approximately 30 minutes, three times a day.

2.2 Postpartum disorders and reproductive management

During the prepartum period (30 days before expected date of calving) pregnant cows were moved to grazing paddocks of Tifton-85 with shade, TMR and water *ad libitum*. During calving, cows were observed and the type of calving was classified as normal, assisted or abortion. Twin births were also recorded. Normal calving needed no interference by humans while assisted calving needed some type of human interference to get a vaginal delivery. Abortion was considered when calving

happened at an unexpected time, between pregnancy day 42-260. Cows that had an abortion were excluded from the trial. Data from clinically healthy multiparous cows with a normal gestation length and a single and live calf were analyzed. Stillbirth is defined as calf mortality shortly before, during, or shortly after parturition [14]. Caesarean section and fetotomy were not performed during the period.

Animals that showed nonuterine disorders during the transition period were not included in the analyses. The uterine disorders evaluated during the puerperium were RP, metritis and clinical endometritis (CE). RP was considered when the cow did not totally eliminate the fetal membranes within the first 12 hours after calving. Metritis (ME) was characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent uterine discharge, which often has a fetid odor. In the farm records RP and ME data were combine. CE was defined by the presence of purulent vaginal discharge containing more than 50% of pus, as analyzed once by Metricheck®, an involuted uterus at transrectal palpation and no clinical systemic signs diagnosed between 21 and 35 DPP [15]. Diagnosed with one of the three uterine disorders in the trial (RP, ME or CE) were treated with Ceftiofur I.M. (1.0 mg/kg of body weight) with a single injection per day for 3 days and Meloxicam I.M. or I.V. (0.5 mg/kg of body weight) once a day during 3 days.

The voluntary waiting period (VWP) established by the farm was 40 days. After this period, cows were evaluated by ultrasonography equipped with a rectal linear transducer of 7.5 MHz (DP3300vet®, Mindray) to evaluate uterine and ovarian conditions. Cows considered healthy (without any uterine disorder), with body condition scores (BCS) greater than 2.5 according to the scale proposed by Edmonson et al. [16] (1 = very skinny and 5 = obese) were submitted to the following timed artificial insemination (TAI) protocol: day 0: insertion of a slow-release intravaginal device containing 1.9 g of progesterone (CIDR®, Zoetis), application of 2.0 mg (2.0 ml) of estradiol benzoate i.m. (Gonadiol®, Zoetis), and 0.25 µg (1.0 ml) of GnRH analog lecirelin i.m. (Dalmarelin®, MSD); day 7: application of 25 mg (5.0 ml) of dinoprost tromethamine i.m. ($PGF_{2\alpha}$ Lutalyse®, Zoetis); day 9: intravaginal device withdrawal, application of 1.0 mg (0.5 ml) of estradiol cypionate i.m. (ECP®, Zoetis) and 25 mg (5.0 ml) of $PGF_{2\alpha}$ i.m. (Lutalyse®, Zoetis); day 11: TAI was performed in all treated animals. The use of estradiol in TAI programs is a legal practice in Brazil. Pregnancy diagnosis was performed ± 35 days after TAI by ultrasound. The cows detected in estrus before the expected date of pregnancy diagnosis were inseminated 12 h after estrus detection. The cows that did not return to estrus after TAI and were not pregnant were resynchronized using the same TAI protocol.

2.3 Statistical analyses

Statistical analyses were performed using SAS Studio (SAS Institute Inc., Cary, NC). Distributions and normality were assessed using the Univariate procedure. Normality was visually assessed using the Kolmogorov Smirnov method. A generalized linear mixed model with the GLIMMIX procedure, was used to assess the occurrence of uterine disorders as the binary dependent variable, using logistic regression with type of calving (normal vs. assisted) as independent variables. The number of AI per conception were used as a dependent variable and assessed with ANOVA using a generalized linear mixed model, fitted using the GLIMMIX procedure of SAS with a Gaussian distribution and an identity link function, with type of calving (normal vs. assisted) as an independent variable. Also, a generalized mixed model with the GLIMMIX procedure was used to assess the pregnancy rate at 150 DPP, as the binary dependent variable, using logistic regression with type of calving and occurrence of uterine puerperium disorders as independent variables. Only variables with a P-value ≤ 0.15 were retained in the final model. Statistical significance was defined as $P \leq 0.05$.

2.4 Animal Rights Declaration

All animal procedures in this research were conducted according to the Ethical Principles in Animal Experimentation, approved by the Committee of Ethics in the Use of Animals (CEUA) of the Federal University of Uberlândia (UFU), protocol number 003/17.

3. Results

A total of 825 calvings were recorded, of which 7 were stillbirths (0.85%) and 17 were twins (2.06%). A total of 801 calvings of a single and live calf were analyzed, of which 766 (95.63%) were normal and 35 (4.36%) were assisted. The overall occurrences were 10.24% (82/801) of RP/ME, 12.86% for CE and 5.12% for RP/ME and CE. Most of the cows that had normal calving had a healthy postpartum period, while among the cows that had assisted calving, the majority had uterine disorders (Table 1).

Table 1. Uterine disorders (retained placenta/metritis and/or clinical endometritis) occurrence according to type of calving (normal vs. assisted).

Type of calving (n)	Healthy	Uterine disorders occurrence
Normal (766)	73.89 % (566) [a]	26.11 % (200) [b]
Assisted (35)	25.71 % (9)[b]	74.29 % (26)[a]

[a, b] Different superscripts within the same line indicate significant differences ($P < 0.05$). n = number; % = percentage.

The number of AI per conception was similar in cows that had normal or assisted calving ($P = 0.16$; Table 2). There was no evidence of a negative influence of type of calving on the pregnancy rate at 150 DPP of crossbred lactating dairy cows ($P = 0.44$; Table 2).

Table 2. Pregnancy rate at 150 DPP and number of AI per conception according to type of calving (normal vs. assisted) in crossbred dairy cows.

Type of calving (n)	Pregnancy rate at 150 DPP (%)	AI /conception (mean ± SE)
Normal (766)	49.48	2.39±0.08
Assisted (35)	42.86	3.00±0.43
P value	0.44	0.16

n = number; % = percentage, SE = standard error, DPP = days postpartum, AI = artificial insemination.

Healthy cows had a higher pregnancy rate at 150 DPP than cows affected by uterine disorders in the postpartum period (Table 3).

Table 3. Pregnancy rate at 150 DPP according to puerperium uterine disorders occurrence in crossbred dairy cows.

Puerperium condition (n)	Pregnancy rate at 150 DPP (%)
Healthy (575)	51.65[a]
Uterine disorders (226)	42.92[b]

[a, b] Different superscripts indicate significant differences ($P < 0.05$). n = number; % = percentage.

4. Discussion

This prospective cohort study aimed to assess the association between type of calving (normal or assisted) on uterine disorders occurrence and its effects on number of AI per conception and pregnancy rate at 150 DPP in crossbred lactating dairy cows, that calving single live calves. Cows with normal calving were more likely to have a healthy postpartum period than cows with assisted calving. The number of AI per conception was similar in cows that had normal or assisted calving. There was no evidence of a negative influence of type of calving on the pregnancy rate at 150 DPP of crossbred lactating dairy cows. Healthy cows had a higher pregnancy rate at 150 DPP than cows affected by uterine disorders in the postpartum period.

Most of the cows that had assisted calving had uterine disorders. Dairy cows affected by dystocia, twin birth, RP, abortion and/or metabolic alterations are more prone to develop uterine disorders [12]. It was reported that cows with peripartum diseases showed higher prevalence of endometritis (62.5%) with longer interval from the beginning of seasonal breeding program to first insemination and a decreasing number of cows with endometritis been inseminated on day 28 of the program (60.2% for cows without endometritis to 34.9% cows with vaginal purulent discharge) [17].

Endometrial inflammation may be a consequence of uterine contamination acquired during calving. Bacterial endotoxins are associated with prolonged anestrus and the luteal phase of the estrous cycle, while ovarian cysts compromise reproductive efficiency and increase the risk of culling. Gilbert et al. [18] reported that Holstein cows with CE showed an increase of 88 days in the calving to conception interval.

Husnain et al. [19] evaluated the effect of inducing endometrial inflammation on Holstein cows and reported a decrease on pregnancy rates (62%) when compared with cows on the control group (88.2%), also a higher risk of these cows became culling cow in comparison with control group (30.1% and 11.3%, respectively).

The 801 cows with normal or assisted calving showed similar number of AI per conception, and the negative effects of assisted calving on pregnancy rate at 150 DPP was not detected. Other studies have reported that dystocia affects production, fertility and cow and calf morbidity and mortality [7]. Dobson et al. [20] also reported delayed uterine involution, delayed onset of luteal activity postpartum and more abnormal progesterone profiles following dystocia. We analyzed crossbred dairy cows that had been treated for metritis, RP and CE, and the number of AI per conception and percentage of pregnant cows at 150 DPP was not affected by the type of calving. This was probably because crossbred cows are less vulnerable to the stress of assisted calving than purebred dairy cows.

Castro-Montoya et al. [21] affirm that cows with ME and/or CE had higher days open (6% and 9.1% respectively) compared with healthy cows. Higher AI/conception rates were also observed when the cows presented postpartum uterine disorders. The interval between services for those cows with CE and needed more than one service increased 10.2% and an increase of 7.9% for cows with ME.

Rezende et al. [22] have found 13.75% of Holstein cows with RP (40/291) in tropical region, and those cows have longer intervals between calving to conception (166.3 days) compared with cows without RP (139.6 days). For crossbreed dairy herds was reported presence of RP in 14.93% of the cows; and the days open was 46 days longer compared with healthy cows [23]. Nobre et al. [24] also found 12.8% of crossbred cows with RP, and 51 days higher interval between calving to conception compared with cows that didn't present RP. According to a meta-analysis conducted by Fourichon et al. [25], cows affected by RP had a 4 to 10% lower conception rate at first service and an increase of 6 to 12 additional days to conception.

Dairy farms that are efficient in productive and reproductive aspects needs to concentrate all efforts to

make a cow conceive as soon as possible after the end of the VWP and so reach a calving interval close to 12 months [26]. The occurrence of uterine disorders at the postpartum period compromises the achievement of this standards, due to the delay on conception and a reduced pregnancy rate at 150 DPP as a consequence.

5. Conclusion

Crossbred dairy cows with normal parturition were healthier at puerperium than cows with assisted parturition considering the uterine disorders evaluated in post partum period. Type of calving did not negatively impact pregnancy rate at 150 DPP, however, healthy cows showed higher pregnancy rate at 150 DPP.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

Conceptualization: R. M. dos Santos*; Data curation*: L. F. F. Coury, C. C. Campos, G. F. de Moraes e R. M. dos Santos; *Formal analysis*: G. F. de Moraes e R. M. dos Santos; *Methodology*: R. M. dos Santos; *Investigation*: L. F. F. Coury e C. C. Campos; *Project administration*: R. M. dos Santos; *Visualização:* N. S. Reis; *Resources*: R. M. dos Santos; *Supevision*: R. M. dos Santos; *Writing (original draft):* L. F. F. Coury, C. C. Campos e G. F. de Moraes; *Writing (review & editing)*: N. S. Reis.

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