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Uterine inflammation and fertility of beef cows subjected to timed AI at different days postpartum

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ABSTRACT

The aim of this study was to evaluate the uterine health and fertility of postpartum beef cows subjected to timed AI (TAI) protocols at different days from calving. Lactating Nelore cows (*Bos indicus*; $n = 244$) were used in this study. The TAI protocols were initiated between 20 and 60 days postpartum (DPP). Cows were subjected to an estradiol-progesterone based TAI protocol. The cows were divided into three groups according to the days postpartum at the time the hormonal treatment was initiated: 1) Early ($n = 64$), cows between 20 and 30 DPP; 2) Middle ($n = 115$), cows between 31 and 45 DPP; and 3) Late ($n = 65$), cows between 46 and 60 DPP. At Day 0 of the protocol, endometrial cytobrush samples were collected. Slides for polymorphonuclear (PMN) cell counting were prepared and cytobrush was used for RNA extraction and analysis of relative abundance of *il1, il6*, *il8*, and *tnf* mRNA. Cows from the Early group had less ($P < 0.05$) pregnancies per AI (P/AI) than cows from Middle and Late groups; 29.7% (19/64), 45.2% (52/115), and 52.31% (34/65), respectively. Accordingly, the Early group had a greater ($P < 0.05$) proportion of PMN cells in the uterus than Middle and Late groups; 9.0%, 3.8%, and 2.2%, respectively. Relative abundances of *il1* and *il8* mRNA were greater ($P < 0.05$) in the Early group than Middle and Late groups. These results indicate that beef cows subjected to TAI protocols early postpartum (< 30 DPP) are less likely to become pregnant, which is associated with increased inflammation and relative abundance of mRNA for the proinflammatory cytokines *il1* and *il8*.

1. Introduction

The use of artificial insemination (AI) protocols for which there is a combining of progesterone and estrogens to improve AI submission rates provides consistent conception results (~50%) in postpartum beef cows (Sales et al., 2012). This reproductive technology has made it possible to AI suckled beef cows without the need to detect estrus (Baruselli et al., 2004). The most common

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timed AI (TAI) protocol for beef cows maintained in tropical areas consists of an intravaginal progesterone insert, estradiol benzoate (EB) to induce synchronous ovarian follicular wave emergence, prostaglandin F2a analogues (PGFs) to induce luteolysis, and an ovulatory stimulus using an ester of estradiol (benzoate or cypionate).

Several studies have been performed to evaluate the factors that affect the fertility of postpartum cows submitted to TAI protocols, such as: hormone concentration, duration of progestagen treatments, time and dose of PGF2 alpha (PGF) injection, ovulation induction agent, estrous cyclicity status, parity, ovarian response, use and dose of eCG, proportion of cows displaying estrus before AI, body condition score (BCS) of the cows, among others (Meneghetti et al., 2009; Sa Filho et al., 2009, 2011; Ayres et al., 2014; Pfeifer et al., 2017). In none of these previous studies was there an evaluation of the effect of uterine health on the fertility of postpartum beef cows subjected to TAI.

The complete postpartum uterine involution is necessary to promote the successful establishment of a pregnancy. The effects of age and milk yield (Fonseca et al., 1983), dystocia (Sheldon and Dobson, 2004), hypocalcemia, metritis and endometritis (Fonseca et al., 1983; Sheldon, 2004; Roche, 2006), among others, can negatively affect uterine involution and the capacity to support pregnancy. After 3 weeks postpartum, the sloughing of necrotic tissue and hemorrhaging have ceased. Both, the weight of the uterus and the diameter of the previously pregnant horn have decreased more than 80% during this period. Grossly, both the weight and the size of the uterus have become static by 40 days postpartum and the uterine epithelium has been completely repaired (Call, 1989).

Endometritis is either uncommon in beef cows, or has a limited impact on subsequent reproductive performance (Santos et al., 2009). Dairy cows with persistent subclinical endometritis, however, were at substantially increased risk of failure to become pregnant before the end of the breeding season (Gilbert et al., 2005). Subclinical endometritis (SEM) is an inflammatory condition of the uterus in the absence of clinical signs, and is characterized by the infiltration of polymorphonuclear cells (PMN, mainly neutrophils) in the endometrium, resulting in a significant reduction of the reproductive performance (Kasimanickam et al., 2004; Sheldon et al., 2009; Ricci et al., 2017). The SEM can be as great as 26% in occurrence between 40 and 60 days postpartum (DPP) in dairy cows (Cheong et al., 2011), and 23% of occurrence after 50 DPP in beef cows (Ricci et al., 2015). Furthermore, associated with the infiltration of PMN cells in the uterus of dairy cows during puerperium, numerous proinflammatory cytokines and chemokines, including Interleukin (IL)-1β, IL-6, and IL-8 and tumor necrosis factor-α (TNF-α) are produced. Although the changes in gene expression of these cytokines are well characterized in dairy cows (Galvao et al., 2011; Ghasemi et al., 2012), there are few reports for beef cows (Foley et al., 2012). The effect of uterine inflammatory secretion in postpartum beef cows raised extensively and under tropical conditions, therefore, is unknown and information in this regard is lacking.

In healthy cows, uterus reparation is not complete until the sixth week postpartum (Call, 1989; Dadarwal et al., 2017; Sheldon, 2004). Considering the need for intensive breeding in tropical cattle production systems, TAI protocols are conventionally initiated as early as 30 to 60 days postpartum (Sa Filho et al., 2009; Vasconcelos et al., 2009; Sa Filho et al., 2010, 2011; Sales et al., 2011, 2012; Pfeifer et al., 2014). Results of all these studies indicated that there were acceptable P/AI ranging from 46% to 65%. In few studies, however, has there been a comparison of the effect of days postpartum on P/AI and in none of the previous studies was there an evaluation of the relationship between uterine health and fertility to determine the optimal postpartum period to the onset of a TAI protocol in beef cows. Setting a minimum DPP, therefore, to include postpartum beef cows in TAI programs can improve reproductive efficiency.

Based on these considerations, the objective of the present study was to evaluate the uterine health and fertility of beef cows subjected to TAI protocols at different days in the postpartum period. The hypotheses was tested that beef cows subjected to TAI early after parturition would have a greater number of inflammatory cells and cytokines in the uterus and be less likely to become pregnant.

2. Materials and methods

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

2.1. Animals, hormonal treatments and sampling

Multiparous lactating Nelore cows (*Bos indicus*; $n = 244$), 4 to 10 years old, from two comercial beef farms in Rondônia – Brazil, were used in this study. All cows were maintained on *Brachiaria brizantha* pasture and given mineralized-salt and free access to water. The TAI protocols were initiated between 20 and 60 days postpartum (DPP). Cows were given 2 mg of estradiol benzoate (Bioestrogen®, Biogénesis-Bagó, Curitiba, Brazil) intramuscular (i.m.) and received an intravaginal progesterone-releasing device (1.9 g progesterone, CIDR®, Pfizer Animal Health, São Paulo, Brazil) to synchronize follicular wave emergence on Day 0. The CIDR was removed and cows were given 150 µg D-Cloprostenol i.m. (PGF2α-analogue; Croniben®, Biogénesis-Bagó, Curitiba, Brazil), 1 mg of ECP im (E.C.P.®, Pfizer, Cravinhos, Brazil), and 300 IU of eCG (Novormon®, Syntex, Buenos Aires, Argentina) i.m. on Day 8. All cows were TAI 48 h after CIDR removal.

Semen from two Nellore bulls at each farm was used for TAI and was equally distributed among treatments. The AI procedures were performed by one experienced technician at each farm. Serum β-hydroxybutyrate (BHB) concentrations were measured on Day 0 with a hand meter device (TD - 4235®; Ketovet, MG, Brazil).