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Associations between resumption of postpartum ovarian activity, uterine health and concentrations of metabolites and acute phase proteins during the transition period in Holstein cows

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ABSTRACT

The resumption of ovarian activity, uterine health, severity of the negative energy balance and the synthesis of inflammatory mediators during the transition period in dairy cows are interrelated. Therefore, the aim of this study was to evaluate the association between the resumption of postpartum ovarian activity and the percentage of polymorphonuclear (PMN) cells in endometrial cytology, lipid mobilization and the secretion of acute phase proteins. For this study, 20 multiparous Holstein cows were used. Blood samples that were collected from 21 d before calving to 44 d in milk (DIM) were analyzed for serum glucose, non-esterified fatty acids (NEFA), insulin, haptoglobin, albumin, paraoxonase and progesterone. Endometrial cytology was performed at 37 ± 2 DIM to evaluate the percentage of PMN cells in the uterine flushing. Cows were divided into two groups: (1) ovulatory cows ($n = 12$), which returned to ovarian activity by 44 ± 2 DIM; and (2) anovulatory cows ($n = 8$), which did not resume ovarian activity during this period. Ovulatory cows had a lower ($P = 0.05$) percentage of PMN cells in endometrial cytology than anovulatory cows (26.3 ± 8.3% *vs.* 53.4 ± 16.9%, respectively). Ovulatory cows had higher serum albumin during the pre- ($P = 0.03$) and postpartum periods ($P = 0.01$), and tended to have lower haptoglobin concentrations in the prepartum period ($P = 0.07$) and higher paraoxonase activity in the postpartum period ($P = 0.09$). In conclusion, cows that resumed ovarian activity early in the postpartum period had higher albumin concentrations in the peripartum period, which were associated with a lower percentage of uterine PMN cells.

1. Introduction

Only approximately 50% of healthy dairy cows ovulate the first dominant follicle within 3 weeks after calving (Kawashima et al., 2007). A healthy postpartum dairy cow can be defined as one that has resolved uterine involution,

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ovulated a dominant follicle early postpartum and continues to have a normal estrous cycle at regular intervals, coupled with homeostatic concentrations of insulin, IGF-I and glucose (Roche, 2006; Walsh et al., 2011). A delayed first postpartum ovulation is usually associated with a pronounced negative energy balance (NEB) in the early postpartum period (Butler, 2003), although other factors are also important (Santos et al., 2009). It is well known that cows that resume ovarian function early after calving are most likely to become pregnant in the first service (Staples et al., 1990). In dairy cows, to achieve a satisfactory reproductive performance during this critical period, it is necessary that the female ovulates a competent oocyte and provides a suitable uterine environment for fertilization and embryonic and fetal development (Pursley and Martins, 2011). Therefore, factors that affect the early resumption of ovarian cycles during the postpartum period have an impact on the fertility of dairy cows and should be further investigated.

In addition to peripartum metabolic events that may affect reproductive performance, uterine contamination may also contribute to decreased conception rates in lactating dairy cows. In total, 80 to 100% of these cows have uterine bacterial contamination during the first weeks after calving (Sheldon et al., 2006). The presence of pathogenic bacteria in the uterus is associated with endometrial inflammation and purulent vaginal discharges (Sheldon et al., 2009), which increase the amount of polymorphonuclear (PMN) defense cells in the endometrium. Most cows recover naturally from these postpartum uterine infections, however, at least 20% of these cows have a persistent infection 3 weeks after calving and develop endometritis. Dairy cows that develop endometritis during the postpartum period have reduced conception at first insemination and increased days open (Fourichon et al., 2000).

During the peripartum period, the increased release of inflammatory mediators, which are called acute phase proteins (APPs) has also been associated with decreased reproductive performance (Wira and Fahey, 2004). Some negative APPs synthesized in the liver, such as paraoxonase (PON) and albumin, have their serum concentrations reduced due to the typical inflammatory response, which is induced by parturition (Bionaz et al., 2007; Fleck, 1989). Conversely, the serum concentration of the positive APP haptoglobin (Hp) increases during the inflammatory process. Several studies have demonstrated increased haptoglobin and decreased albumin and paraoxonase concentrations in cows with postpartum uterine infections, even before calving (Sheldon et al., 2001; Turk et al., 2004, 2005; Huzzey et al., 2009; Burke et al., 2010; Schneider et al., 2013). Cows with bacterial infections in the reproductive tract have slower growth of the dominant follicle, lower plasma concentrations of estradiol and progesterone and are less likely to ovulate (Sheldon et al., 2002; Williams et al., 2007). Therefore, these results indicate that uterine health might be associated with the secretion of APPs and with the timing of the first postpartum ovulation.

Based on these considerations, we hypothesized that cows resuming ovarian activity earlier during the postpartum period have a reduced percentage of PMN endometrial cells in the uterus, and that these conditions are reflected by the inflammatory status, which is indicated by the serum concentrations of APPs. Therefore, the aim of this study was to evaluate the association of the timing of the first postpartum ovulation with the following: (1) the percentage of PMN endometrial cells in the uterine flushing, (2) lipid mobilization, and (3) the secretion of APPs during the transition period of dairy cows that are maintained in a semi-extensive management system.

2. Materials and methods

2.1. Cows and experimental design

The experiment was conducted between December 2011 and June 2012 in a commercial farm in Rio Grande, RS, Brazil (32° 16' S, 52° 32' W). The Ethics and Animal Experimentation Committee from the Federal University of Pelotas, under the registration number 4551, approved all procedures. The cows were selected for the study based on the number of lactations (≥3 lactations), the average production, which was adjusted for 305 days during the previous lactation (≥7.000 kg), and a negative history of any clinical disease during the last lactation. Initially, forty ($n = 40$) multiparous Holstein dairy cows (≥3 lactations), with an average body condition score (BCS) of 3.1 ± 0.4 and a body weight (BW) of 629.8 ± 42.7 kg, were enrolled in the study. Twenty ($n = 20$) cows were diagnosed with one or more pathological event, including dystocia, retained placenta, mastitis, laminitis and hypocalcemia, and were excluded from the experiment. The cows were maintained in a semi-extensive management system, which was based on pasture and concentrate supplementation after each milking. Milking was performed twice a day, at 12 h intervals.

The cows were divided into two groups according to the return of luteal activity during the postpartum period as follows: (1) cows that resumed ovarian activity before 44 days in milk (DIM) (ovulatory cows), which had progesterone concentrations that were higher than 1 ng/mL in at least two consecutive blood samples that were collected between 16 and 44 DIM, and (2) cows that did not resume ovarian activity before 44 DIM (anovulatory cows), which had no increase in progesterone concentrations above 1 ng/mL during the same period (Stevenson and Britt, 1979).

2.2. Blood sampling and biochemical and hormonal analysis

Blood sampling was performed on days −21, −14, −7, −3 before calving, at calving (0 DIM) and at 3, 6, 9, 16, 23, 30, 37 and 44 DIM. To evaluate the resumption of ovarian activity, blood sampling for the measurement of serum progesterone (P4) was performed at 16, 23, 30, 37 and 44 DIM. Blood was collected by coccygeal venipuncture using vaccutainer tubes. The blood samples were collected in tubes that contained clot activator (16 × 100 mm, 10 mL Vacuplast®, Shandong, China) and sodium fluoride (13 × 75 mm, 4 mL Vacuplast®, Zhejiang, China), centrifuged within 30 min after collection at $1.35 \times g$ for 15 min. For all blood variables, except for progesterone, the