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Effect of bovine somatotropin injection in late pregnant Holstein heifers on metabolic parameters and steroidogenic potential of the first postpartum dominant follicle

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ABSTRACT

The aim of this study was to determine the effect of pre-partum injections of bovine somatotropin (bST) in dairy heifers on metabolic markers and the steroidogenic potential of the first postpartum dominant follicle. Heifers were assigned to two groups: bST (**ST**; n = 29), that received two doses of bST (500 mg/dose) at −28 and −14 days relative to calving; and control (**CTL**; n = 30), that did not received bST. Follicular development was monitored via ultrasound every 3 days starting at 8 days in milk (DIM) in a subset of 20 heifers until the day the first large follicle reached a diameter of 16 mm. From these cows follicular fluid was aspirated and the follicular cells recovered (ST; n = 8 and CTL; n = 10). Blood samples were collected weekly for all heifers. Follicular fluid IGF-I concentrations of the first postpartum dominant follicle was higher (P = 0.05) in ST (87.1 ± 7.7 mg/mL) than CTL cows (64.3 ± 6.8 mg/mL). Also, E2 concentration in the follicular fluid was higher (P = 0.02) for ST (199.7 ± 55.9 ng/mL) than CTL cows (74.5 ± 37.7 ng/mL). The expression of *LHCGR* and *STAR* mRNA in follicular cells was higher (P < 0.05) in ST than CTL cows. Nonetheless, *HSD3B, P450scc, P450c17, IGFr* and *CYP19A1* mRNA expression was not different between groups (P > 0.05). Serum IGF-I concentration was higher in ST treated heifers during the pre-partum period (P = 0.01) and no difference was observed in the postpartum period (P = 0.19). In conclusion, pre-partum bST treatment in dairy heifers increased intrafollicular IGF-I and expression of *LHCGR* and *STAR* mRNA in follicular cells of the first postpartum dominant follicle. These changes were associated to increased intrafollicular and serum E2 concentration, which can potentially increase the chance of ovulation of the first follicular wave.

1. Introduction

During the early postpartum period of dairy cows the energy demands increase and exceed the intake capacity, resulting in a period of negative energy balance (NEB) [1,2]. The duration and the intensity of the postpartum NEB is negatively associated with reproductive performance. Cows that experience a more intense NEB have delayed return of postpartum ovarian activity and, consequently, take longer to conceive [3–6]. In this regard, the more estrous cycles before the time of first insemination, the higher the probability of pregnancy [7–9]. Therefore, the use of strategies that decrease the intensity of the early postpartum NEB, can anticipate the time to first ovulation and may also reduce time to conception.

Several strategies have been used to improve the metabolic condition of cows in the transition period and thus the reproductive performance and overall postpartum health. Administration of low doses of bovine somatotropin (bST) in the periparturient period have been associated with beneficial effects on the physiological

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adaptation and liver function of high-producing dairy cows [10–14]. bST regulates nutrient partitioning, and is traditionally used at the postpartum period to increase milk production [10,15,16], also having direct effects in the mammary tissue mediated by insulin-like growth factor I (IGF-I) [17,18]. In this regard, bST injection during the peripartum period has been shown to increase pre and post-partum IGF-I concentration [12,19], milk production [12,19,20], postpartum dry matter intake [11,14], post-partum glucose [14,21] and to reduce post-partum fat mobilization [14], reducing non-esterified fatty acids (NEFA) mobilization and hepatic β-hydroxybutyrate (BHBA) formation [12,14]. This suggests that prepartum bST injection can improve metabolic adaptation during the transition period in dairy cows. However, its effects on the development of the first postpartum follicular wave and ovulation are still scarce.

Most of the positive effects of bST are exerted through the actions of IGF-I [17]. Increased plasma concentration of IGF-I is beneficial for follicle development, since it acts as a modulator of gonadotropin action in the ovary, stimulating steroidogenesis [22], granulosa and theca cell proliferation and differentiation [23] and preventing follicular apoptosis [24]. A dominant follicle (>10 mm) can be present as early as 8 days post-partum (DPP) in dairy cows and by 21 DPP a considerable proportion of cows have already ovulated during this first postpartum follicular wave [25–27]. Cows that ovulated the first postpartum wave have higher serum IGF-I concentration in the prepartum and early postpartum period, and this may be due to the fact that higher serum IGF-I reflects a less intense NEB [25,28]. Additionally, the number of recruited follicles per wave is increased in dry cows or heifers treated with bST [29–31]. Also, this effect on follicle recruitment persisted for at least 21 days after the end of the treatment of primiparous Holstein cows that received seven bST injections between 120 and 180 days postpartum [31]. This evidence suggests that prepartum bST treatment may reflect on ovarian activity later on the postpartum period, when a faster return to ovulatory activity is critical for improving reproductive performance. In this sense, pre-partum bST treatment has been shown to decrease the time from calving to return ovarian activity [12], although the mechanism involved in this process is not yet fully understood. Therefore, understanding the mechanisms associated with increased steroidogenesis in the first postpartum follicular wave it is essential in order to design better therapies in the future aiming to improve reproductive efficiency in dairy cows.

The aim of this study was to determine the effect of pre-partum injections of bST on serum markers of energy, protein and fat metabolism (IGF-I, BUN and NEFA), milk production, and the hormonal concentration (estradiol and progesterone) and gene expression (through evaluation of hormone receptor and steroidogenic enzymes - LHCGR, STAR, *HSD3B, P450scc, P450c17, IGFr* and *CYP19A1*) during the first postpartum follicular wave in primiparous Holstein cows.

2. Materials and methods

All procedures performed in this experiment were approved by the Committee for Ethics in Animal Experimentation from the Federal University of Pelotas (Pelotas, RS, Brazil).

2.1. Location and experimental design

For this study 59 late pregnant Holstein heifers from a commercial dairy herd in southern Brazil (32° 16′ S, 52° 32′ W) were used. The heifers had a mean body condition score (BCS) of 2.9 ± 0.3 (ranging from 2.5 to 4.0) at the beginning of the experiment. All calving occurred in a 40 days interval during the winter season. Heifers were randomized according to the date of expected calving and BCS to ensure that these factors had minimal chance of influencing the outcome variables of this study.

At −28 ± 1 days relative to calving (262 ± 3 days of pregnancy), heifers were assigned to one of two groups: bST (**ST**; n = 29) that received two doses of bST (Lactotropin®, 500 mg/cow s.c., Elanco, Sao Paulo, Brazil) at −28 ± 1 days and −14 ± 1 days relative to calving, and Control (**CTL**; n = 30) that did not received bST application.

All heifers were managed under the same conditions and nutritional regimen. The far-off diet from 60 to 30 days before expected calving was based in rice straw and native pasture *ad libitum*. The close-up diet from 30 days before the expected calving until parturition was also based in rice straw and native pasture *ad libitum*, but also included 10 kg of sorghum silage, 3 kg of a concentrate mix [23.2% of soybean meal, 9.27% of corn bran, 9.7% of soybean hulls, 4.73% of mineral BCA (BCA Preparto, Tortuga, Mairinque, SP, Brazil), 1.7% of mineral NNBI + MB (Novo Bovigold, Tortuga, Mairinque, SP, Brazil) and 2.85% of calcitic limestone]. The lactation diet from calving to 30 days in milk (DIM) was based in ryegrass pasture and white clover pasture *ad libitum*, 10 kg of sorghum silage, and 10 kg of the same concentrate mix for close-up diet except for the mineral BCA.

2.2. Ultrasonographic evaluations and follicular aspiration

Transrectal ultrasonography was performed using a 7.5-MHz linear array probe (Welld® Wed-3000 V, Shenzhen, Guangdong, China), in a randomly selected subset of heifers from each group (n = 10/group). Ultrasound examination of the ovaries was performed every 3 days from 8 DIM up to the day that the largest follicle on the ovaries reached a diameter close to 16 mm (which is the average diameter of the follicle that may be ovulated during the first postpartum wave in Holstein heifers according to Butler et al. [25] and Cheong et al. [27]). At this moment (ST = 13.16 ± 1.9 DIM and CTL = 14.26 ± 1.7 DIM) follicular fluid from the largest follicle of eighteen heifers (ST; n = 8 and CTL; n = 10) was aspirated. The follicle was aspirated by ultrasound-guided transvaginal follicular aspiration. We were not able to aspirate two heifers from the ST group due to difficulties with the aspiration procedure.

An ultrasound scanner (Welld Wed-3000 V) equipped with a 7.5-MHz probe was used for the follicle aspiration procedure. The ultrasound probe was enclosed within a custom-made handle. The ovary containing the largest follicle was manipulated toward the ultrasound probe to be inspected and measured before aspiration. The 18-gauge aspiration needle was guided through the stroma of the ovary and into the follicle and the contents was aspirated with a 10 mL syringe. The follicular fluid was centrifuged and the clear supernatant was frozen at −80 °C for subsequent analysis. The resulting pellet of follicular cells was immediately transferred to a new tube and homogenized with Trizol reagent (Life Technologies, Inc., Grand Island, NY, USA), frozen in liquid nitrogen and stored at −80 °C. Only estrogen active follicles (ST n = 6 and CTL n = 8), selected based on estradiol (E2):progesterone (P4) ratio higher than 1 in the follicular fluid [27], were used for gene expression and follicular fluid content analysis.

2.3. Hormones and metabolites analyses

Blood samples were collected weekly from −28 ± 1 days before expected calving until parturition, at calving and at days 3, 6, 14 and 28 ± 1 via puncture of the coccigeal vein or artery in one tube without anticoagulant to evaluate P4, NEFA, IGF-I and BUN concentrations. For E2 analyses blood samples were collected every 3 days in a subset of heifers along with ultrasound examination (ST; n = 10 and CTL; n = 10). Blood samples were collected in the interval