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The effect of energy balance on the transcriptome of bovine granulosa cells at 60 days postpartum

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ABSTRACT

Dairy cows expend great amounts of energy during the lactation peak to cope with milk production. A state of negative energy balance (NEB) was suggested as a cause for the suboptimal fertility observed during this period, *via* an interaction with ovarian function. The objective of this study was to identify the impact of NEB on gene expression in granulosa cells of dairy cows at 60 days postpartum and to suggest a potential treatment to improve ovarian function. Dairy cows at 60 days postpartum from 10 typical medium-sized farms were synchronized using a single injection of prostaglandin. Dominant follicles were collected 42 hours later by transvaginal aspiration. Blood concentrations of beta-hydroxybutyrate (BHB) on the day of aspiration were used to classify animals into two groups: severe NEB (high BHB, n = 12) and mild NEB (low BHB, n = 12). The transcriptomes of granulosa cells from both groups were contrasted using microarrays, and the differentially expressed genes were analyzed using Ingenuity Pathway Analysis to identify affected functions and potential upstream regulators. Genes linked with cellular organization (*KRT4* and *PPL*), proliferation (*TACSTD2*), and fatty acids metabolism (*VNN2*) were downregulated in granulosa cells from animals with severe NEB. Several genes linked to decitabine, a hypomethylating agent, and with beta-estradiol, were downregulated in the severe NEB group. Numerous genes linked to vitamins A and D were also downregulated in this group of cows, suggesting a potential deficiency of these vitamins in dairy cows during the postpartum period. This study supports the idea that energy balance has an impact on follicular dynamics which could be detrimental to resumption of fertility after calving.

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1. Introduction

Dairy production is an important aspect of the food industry in many countries. As the pregnancy and birth of a calf are essential to the onset of milk production in the cow, the dairy industry requires fertile animals. However, the past decades have seen a noticeable drop in the fertility of Canadian dairy cows, mainly for the Holstein breed. This trend is worrisome for producers, as it is associated with additional expenses and lower profits. The main problems observed are longer intervals between calving and first insemination [1], decreased conception rates [2], abnormal estrus [3], and increased pregnancy losses [4]. These problems considerably reduce the longevity of dairy cow herds: In 2013, 8.2% of dairy cows registered in Canada were culled for reproductive reasons (Canadian Dairy Information Centre). No single factor was proven to be the cause of the declining fertility in dairy cattle, but increased lactation demands was suggested to play a major role. Global conception rates have increased during the past few years, and the milk yield per animal also increased over the same period [5]. However, although previous studies highlighted relations between milk production and several aspects of cattle reproduction, they were not able to report a direct relationship between milk yield and fertility [6,7].

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During the postpartum period, dairy cows experience a period of negative energy balance (NEB) and need to mobilize their body reserves to cope with the energy demand of lactation [8]. The amount of energy required for milk production increases quickly after calving, whereas the increase in dry matter intake lags behind [9]. Animals selected for milk production tend to partition more energy toward milk rather than restoring body reserves [10,11] and are therefore more susceptible to experience a prolonged period of NEB. Fertility in heifers and beef cows has not declined in a similar way as fertility in lactating cows [12–14], supporting the hypothesis of an excessive metabolic stress during lactation. However, energy balance status may affect reproduction [15] more than milk yield, as it induces metabolic stress.

During periods of fasting or of high energy demand such as lactation, body energy reserves are mobilized and lipid metabolism is activated. The released fatty acids are oxidized by the liver to produce ketone bodies, which can transport energy from the liver to other tissues. The concentration of these molecules in blood serum increases with lipid mobilization. The most abundant ketone body is beta-hydroxybutyrate (BHB), and its blood concentration is an indicator of ketosis in cattle, with subclinical ketosis ranging from 1200 to 1400 μmol/L [16]. Cows with a higher body condition score (BCS) at calving are more likely to experience ketosis [17] because they eat less than thin cows and will therefore need to mobilize more body reserves to cope with the energy demand of lactation [18,19]. In dairy cows, high concentrations of BHB were linked to periparturient diseases [20].

The follicular environment greatly influences the competence of the oocyte [21] and, therefore, ultimately contributes to embryo quality. Granulosa cells' transcriptome is known to be altered by numerous aspects of follicular development, including growth stage [22,23]. *In vitro* studies have shown that some blood metabolites are linked to energy balance, such as nonesterified fatty acids and BHB, and can influence bovine granulosa cell functions [24–26]. Our hypothesis is that energy balance has an impact on bovine granulosa cells' transcriptome. The objective of this study was to analyze the transcriptomic profiles of granulosa cells from dairy cows with more or less severe NEB at 60 days postpartum, to identify key genes and pathways connecting energy balance and follicular development.

2. Materials and methods

2.1. Animals

Lactating Holstein cows were selected from 10 medium-sized dairy farms with 75 to 300 animals. Several small farms, with dietary and management differences, were preferred to a single large farm as a way to allow broader application of the conclusions. Animal diets and management of the farms were taken into consideration, and animals were paired accordingly for all contrasts. All samples were collected during the winter of 2013 to 2014. The farm managers were asked to provide milk recording data for each animal.

2.2. Tissue collection

Cows between 50 and 70 days postpartum were given a single dose of prostaglandin by intramuscular injection. Caudal epidural anesthesia was done through an intervertebral injection of lidocaine, and an ultrasound was performed through rectal palpation. Ultrasound was used to measure diameter of the dominant follicle 42 to 44 hours after PGF injection. The follicles were collected by transvaginal aspiration, and a washing procedure was used to gather a maximum number of granulosa cells from each follicle. After the collection of follicular fluid and while the needle was still in the follicle, the collection syringe was unscrewed from the needle tube and replaced by a new syringe filled with 10 mL of a cold PBS solution (pH 7.1, concentrations in g/L: 0.2 KCL, 0.2 KH_2PO_4, 8.0 NaCl, 1.14 Na_2HPO_4, 1.0 D-glucose [dextrose], 0.11 pyruvate [pyruvic acid], BSA 0.4%). The PBS was then slowly pushed inside to refill the follicle. The PBS syringe was then unscrewed, and the collection syringe was screwed back in place to collect the liquid again. To avoid contamination between samples, a new set of sterile syringes was used for each aspiration. The BCS was evaluated by a veterinarian on the same day on a scale of one to five (five = obese), and blood samples were collected by venipuncture on the tail just after aspiration. The same veterinarian handled all the animals and evaluated all the BCS.

2.3. Processing of samples

The fluid collected from each cow was immediately transferred into a 15-mL RNase-free tube containing 2 mL of a sterile cold PBS-EDTA 60 μM solution. If needed, cold PBS was added to the tube to ensure that the final volume for each sample was 12 mL. The tube was then centrifuged 3 minutes at 5000 rpm at room temperature, and the supernatant was discarded. The pellet was resuspended with 5 mL of cold PBS-EDTA 10 μM and centrifuged a second time at room temperature for 3 minutes at 5000 rpm. The supernatant was discarded again, and 1 mL of RNAlater (Life Technologies Inc., Burlington, ON, USA) was added to the sample tube to stabilize and protect cellular RNA. The tube was kept at 4 °C overnight (for a maximum of 16 hours). The sample was then transferred to a 1.5-mL RNase-free Eppendorf tube and centrifuged 20 minutes at 5000 rpm at room temperature. The supernatant was discarded, and the pellets were stored at −20 °C.

2.4. RNA extraction

To filter out insoluble debris and reduce viscosity, the samples were passed through QIAshredder homogenizer columns (Qiagen, Toronto, ON, USA) before RNA extraction. Total RNA extraction was then performed using the PicoPure RNA Isolation kit (Life Technologies Inc.) under an RNase-free environment and included a DNase 1 digestion (Qiagen) step. The quality and concentration of all RNA samples were assessed with a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA), using RNA 6000 Nano and Pico reagents (Agilent). Samples with an RNA integrity number lower than 6.8 or a concentration lower than 0.75 ng/μL were rejected.

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