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Effects of rumen-protected methionine and choline supplementation on steroidogenic potential of the first postpartum dominant follicle and expression of immune mediators in Holstein cows

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ABSTRACT

Multiparous Holstein cows were assigned in a randomized complete block design into four treatments from 21 d before calving to 30 d in milk (DIM). Treatments were: **MET** [n = 19, fed the basal diet + rumen-protected methionine at a rate of 0.08% (w/w) of the dry matter, Smartamine® M], **CHO** (n = 17, fed the basal diet + choline 60 g/d, Reashure®), **MIX** (n = 21, fed the basal diet + Smartamine® M at a rate of 0.08% (w/w) of the dry matter and 60 g/d Reashure®), and **CON** (n = 20, no supplementation, fed the close-up and fresh cow diets). Follicular development was monitored via ultrasound every 2 d starting at 7 DIM until ovulation (n = 37) or aspiration (n = 40) of the first postpartum dominant follicle (**DF**). Follicular fluid from 40 cows was aspirated and cells were retrieved immediately by centrifugation. Gene expression of *TLR4, TNF, IL1-β, IL8, IL6, LHCGR, STAR, 3β-HSD, P450scc, CYP19A1, IRS1, IGF, MAT1A, and SAHH,* was measured in the follicular cells of the first DF. Cows in CON had higher *TNF, TLR4,* and *IL1-β* mRNA expression (11.70 ± 4.6, 21.29 ± 10.4, 6.28 ± 1.4, respectively) than CHO (2.77 ± 0.9, 2.16 ± 0.9, 2.29 ± 0.7, respectively), and MIX (2.23 ± 0.7, 1.46 ± 0.6, 2.92 ± 0.8, respectively). Cows in CON had higher *IL1*-β expression (6.27 ± 1.4) than cows in MET (3.28 ± 0.6). Expression of *IL8* mRNA was lower for cows in CHO (0.98 ± 0.3) than cows in CON (4.90 ± 0.7), MET (6.10 ± 1.7), or MIX (5.05 ± 1.8). Treatments did not affect mRNA expression of *LHCGR*, *STAR*, *P450scc*, *CYP19A*, *SAHH*, *MAT1A*, or *IL6* however, *3β-HSD* expression was higher for cows in MET (1.46 ± 0.3) and MIX (1.25 ± 0.3) than CON (0.17 ± 0.04) and CHO (0.26 ± 0.1). Supplementation of methionine, choline, and both methionine and choline during the transition period did not affect days to first ovulation or number of cows that ovulated the first follicular wave. Plasma and follicular fluid estradiol and progesterone concentrations were not different among treatments. Methionine concentrations in the follicular fluid of the first postpartum DF was higher for cows in MET (18.2 ± 0.1 μ*M*) than cows in CON (11.1 ± 0.9 μ*M*). In conclusion, supplementing choline and methionine during the transition period changed mRNA expression in follicular cells and dietary methionine supplementation increased plasma and follicular fluid concentrations of methionine of the first postpartum DF in Holstein cows.

1. Introduction

The transition period for a dairy cow has traditionally been defined from three weeks prepartum until three weeks postpartum [1]. In this period, dairy cows often experience negative energy balance (NEB) that induces excessive tissue mobilization, primarily fat but also protein [2]. Amino acids (AA) make up a large proportion of the precursors needed for gluconeogenesis [1]. Moreover, NEB has been shown to have effects on the metabolic status and reproductive performance of dairy cows [3,4]. Perhaps the most

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detrimental impact of NEB on reproductive performance is delayed return to cyclicity [5]. It is well documented that cows with more estrous cycles before their first artificial insemination have higher probability of pregnancy [6]. Therefore, the use of strategies that shorten the time to first ovulation will most likely hasten the time to conception.

The dominant follicle (DF) growth and estradiol (E_2) production are key factors for a successful conception, and their impairment can be attributed to reduced luteinizing hormone (LH) pulses [7] as well as decreased circulating insulin and IGF-I concentrations [2,8]. Furthermore, immune function is also suppressed along the periparturient period [3,9], NEB, and fatty liver syndrome demonstrated to impair peripheral blood neutrophil function [10,11]. It has been proposed that mRNA expression of LH receptor (*LHCGR*) [12] and 3-beta-Hydroxysteroid dehydrogenase (*3β-HSD*) in granulosa cells can play a role in the establishment of follicular selection [13].

A number of reviews have highlighted the importance of nutrition in regulating bovine reproductive efficiency [14–17]. The effects of rumen-protected methionine (RPM) supplementation on reproductive efficiency of dairy cattle have not yet been fully evaluated [18]. Furthermore, methionine is often a limiting AA in early lactation [19] and the reduced availability of methionine in early lactation could decrease the synthesis of phosphatidylcholine and impair lipid metabolism. Increased methionine bioavailability is likely to increase entry of methionine into the 1-carbon metabolism cycle where it is initially converted into *S*-adenosylmethionine (SAM), the major biological methyl donor [20]. Methylation of DNA is an important mechanism for regulation of gene expression. This methylation depends on the availability of methyl donors supplied by AA such as methionine and by compounds of one-carbon metabolic pathways such as choline [21]. It has been established that choline is an essential nutrient for mammals when sufficient methionine is not available in the diet [22,23]. It has been shown that increased rumen-protected choline (RPC) supplementation reduced the esterification of fatty acids (NEFA) in the liver without altering fatty acid oxidation [24], thereby suggesting decreased hepatic triacylglycerol (TAG) accumulation. In addition, RPC supplementation reduced plasma NEFA concentration and NEFA to cholesterol ratio around parturition [25], which could reduce the risk of lipid-related metabolic disorders and improve fertility [6]. Moreover, accumulation of TAG in the liver was positively correlated with higher number of days from calving to first ovulation [26].

Therefore, the objectives of the current study were to determine the effects of supplementing RPM, RPC, and their combination during the transition period on resumption of ovulation of the first DF postpartum, the expression of genes related to the pro-inflammatory process and steroidogenic pathway in the follicular cells of the first DF postpartum in Holstein cows.

2. Materials and methods

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee.

2.1. Experimental design and dietary treatments

A complete data set was obtained and used from a total of 77 pregnant Holstein cows entering their second or greater lactation [parity 3.1 ± 1.2 and body weight (BW) 773 ± 16 kg]. Cows were blocked with regard to lactation number and previous lactation 305-days milk yield to ensure that these variables had minimal chance of influencing the outcome variables of the study.

Detailed procedure is described elsewhere [27]. Briefly, all cows received the same far-off diet from 50 to 22 d before expected calving, close-up diet from 21d before expected calving until parturition, and a lactation diet from calving through 30 DIM. Diets and their respective chemical composition are elsewhere [27].

At − 21 ± 2 d relative to calving, cows were randomly assigned to one of four treatments, given as top-dress on a TMR. The dry matter (DM) of the total mixed ratio (TMR) for the close-up and lactation diets was measured weekly for estimation of daily TMR DM offered. Supplementation with RPM; [**MET;** n = 19; RPM at rate of 0.08% (w/w) of the DM, Smartamine® M (Adisseo, Alpharetta, GA, USA)], RPC; [**CHO;** n = 17; were received 60 g/d choline, Reassure® (Balchem Corporation, New Hampton, NY, USA)], both feed supplements [(**MIX;** n = 21; RPM at a rate of 0.08% (w/w) of the DM (Smartamine® M) and 60 g/d choline (Reashure®)] or no supplementation (**CON;** n = 20). Dosage of RPM was based on Osorio et al. [28]; whereas, CHO was supplied following the manufacturer's recommendations.

2.2. Reproductive management and ultrasonography evaluations

The first postpartum follicular ovulation was monitored in 37 cows (MET, n = 10; CHO n = 8; MIX n = 9, and CON, n = 10) beginning at 7 DIM and then examinations were performed every 2 d until ovulation using transrectal ultrasonography (E.I. Medical Imaging, Loveland, Colorado) with 7.5-MHz linear array probe. At each examination a sketch of each ovary was made, and the diameter and location of follicles >3 mm in diameter were recorded [29]. Ovulation was defined as the disappearance (from one examination to the next) of a previously identified follicle >8 mm in diameter [30] along with the detection of a CL in the same ovary in a further examination.

2.3. Follicular aspiration

Follicular aspiration was performed when the DF reached a diameter of ~16 mm (MET: 13.16 ± 0.89, CHO: 14.27 ± 0.90, MIX: 12.73 ± 0.85, CON: 13.07 ± 0.79 DIM); average diameter of the follicle to ovulate during the first postpartum wave in Holstein cows [31]. At that point, follicular fluid from 40 cows (MET, n = 9; CHO, n = 9; MIX, n = 12; CON, n = 10) was aspirated. The DF was aspirated by ultrasound-guided transvaginal follicular aspiration. Briefly, the area above the first intercoccygeal space was clipped and disinfected with an iodine scrub solution and 70% ethanol. Lidocaine (5 mL, 2% lidocaine hydrochloride solution, Hospira, Inc., Lake Forest, IL, USA) was injected into the first intercoccygeal space and time was allowed for the anesthesia to take effect. The vulva and perianal area were cleaned and disinfected with iodine scrub solution.

An IBEX® ultrasound scanner equipped with a 7.5-MHz curvilinear probe was used for the follicle aspiration procedure. The ultrasound probe was enclosed within a custom-made handle. The handle enclosed the probe cord and fixed the head of the probe at a 30° angle relative to the needle guide. The needle guide ended at the base of the probe, just above the probe head. The probe and handle were lubricated with a sterile water-based lubricant and positioned in the vagina slightly posterior to the cervix. The ovary containing the DF was manipulated toward the ultrasound probe to be inspected and measured before aspiration. An 18-gauge aspiration needle was passed through the needle guide of the handle and then through the vagina. The aspiration needle (18 G, WTA, Cravinhos, SP, Brazil) was guided through the stroma of the ovary and into the DF. The follicular fluid was retrieved and frozen at −80 °C for subsequent analysis of hormones. The follicular cells were immediately pelleted by centrifugation, at 4 °C for 10 min at 2000×*g* and the follicular fluid was decanted. The cells were