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Effects of rumen-protected methionine and choline supplementation on the preimplantation embryo in Holstein cows

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ABSTRACT

Our objective was to determine the effects of supplementing methionine and choline during the prepartum and postpartum periods on preimplantation embryos of Holstein cows. Multiparous cows were assigned in a randomized complete-block design into four treatments from 21 days before calving to 30 days in milk (DIM). Treatments (TRT) were MET (n = 9, fed the basal diet + rumen-protected methionine at a rate of 0.08% [w:w] of the dry matter [DM], Smartamine M), CHO (n = 8, fed the basal diet + choline 60 g/d, Reashure), MIX (n = 11, fed the basal diet + Smartamine M and 60 g/d Reashure), and CON (n = 8, no supplementation, fed the close-up and fresh cow diets). Cows were randomly reassigned to two new groups (GRP) to receive the following diets from 31 to 72 DIM; control (CNT, n = 16, fed a basal diet) and SMT (n = 20, fed the basal diet + 0.08% [w:w] of the dry matter intake as methionine). An progesterone intravaginal insert (CIDR) device was inserted in all cows after follicular aspiration (60 DIM) and superovulation began at Day 61.5 using FSH in eight decreasing doses at 12-hour intervals over a 4-day period. On Days 63 and 64, all cows received two injections of PGF2α, and CIDR was removed on Day 65. Twenty-four hours after CIDR removal, ovulation was induced with GnRH. Cows received artificial insemination at 12 hours and 24 hours after GnRH. Embryos were flushed 6.5 days after artificial insemination. Global methylation of the embryos was assessed by immunofluorescent labeling of 5-methylcytosine, whereas lipid content was assessed by staining with Nile red. Nuclear staining was used to count the total number of cells per embryo. There was no difference between TRT, GRP, or their interaction ($P > 0.05$) for embryo recovery, embryos recovered, embryo quality, embryo stage, or cells per embryo. Methylation of the DNA had a TRT by GRP interaction ($P = 0.01$). Embryos from cows in CON-CNT had greater ($P = 0.04$) methylation (0.87 ± 0.09 arbitrary units [AU]) than embryos from cows in MET-CNT (0.44 ± 0.07 AU). The cytoplasmic lipid content was not affected ($P > 0.05$) by TRT or their interaction, but lipid content was greater ($P = 0.04$) for SMT (7.02 ± 1.03 AU) than that in CNT (3.61 ± 1.20 AU). In conclusion, cows in MET-CNT had embryos with lower methylation, and SMT cows had a higher lipid content than CNT. Methionine supplementation seems to impact the preimplantation embryo in a way that enhances its capacity for survival because there is strong evidence that endogenous lipid reserves serve as an energy substrate.

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1. Introduction

Studies over the last 2 decades clearly established the link between nutrition and fertility in ruminants [1–5]. Dietary changes can cause an immediate and rapid alteration in a range of humoral factors that can alter endocrine and metabolic signaling pathways crucial for reproductive function [6,7]. Moreover, periconceptional nutritional environment in humans and other animals is critical for the long-term setting of postnatal phenotype [8]. Restricting the supply of B-vitamins and methionine during the periconceptional period in sheep, e.g., resulted in adverse cardiometabolic health in postnatal offspring [9]. Feeding female mice a low-protein diet during the preimplantation period of pregnancy resulted in a reduction in amino acid (AA) concentration in uterine fluid and serum and attendant changes in the AA profile of the blastocyst [10].

Strategies have been used to improve the reproductive performance of dairy cows through alteration of nutritional status [11,12]. In other species, dietary supplementation with specific AAs (e.g., arginine, glutamine, leucine, glycine, and methionine) had beneficial effects on embryonic and fetal survival and growth through regulation of key signaling and metabolic pathways [13,14].

Methionine is the most limiting AA in lactating cows [15], but supplementation of diets with crystalline methionine has been excluded because free methionine is quickly and almost totally degraded by the microorganisms in the rumen [15]. In contrast, supplementing rumen-protected methionine (RPM) has a positive effect on milk protein synthesis in dairy cows [16–18]. Although the role of methionine in bovine embryonic development is unknown, there is evidence that methionine availability alters the transcriptome of bovine preimplantation embryos *in vivo* [19].

The DNA methylation in promoters is an important mechanism for regulation of gene expression and gene silencing. However, DNA methylation in other regions may have a more complex role in regulation of transcription [20–22]. Methylation of the DNA depends on the availability of methyl donors supplied by AAs such as methionine and by compounds of one-carbon metabolic pathways such as choline [21]. Increased methionine bioavailability is likely to increase the entry of methionine into the one-carbon metabolism cycle where it is initially converted into S-adenosylmethionine, the major biological methyl donor [23].

Choline is a major component of phospholipids, and sphingomyelin, a component of acetylcholine that participates directly in neurotransmission [24], affects membrane integrity and alters methylation pathways [25,26]. Early studies evaluating the effect of dietary choline on milk yield and duodenal flow indicated its rapid and extensive rumen degradation before absorption in duodenum [27,28]. Subsequently, numerous studies have evaluated the effects of feeding rumen-protected choline (RPC) on reproduction and health of dairy cows [29,30].

Nonruminants fed diets deficient in methyl donors (e.g., choline and methionine) have hypomethylated DNA [31,32]. These changes occur not only in global methylation [33] but also in the methylation of specific genes [34]. However, effects of methionine in preimplantation embryos are still controversial. Bonilla et al. [35] suggested that extracellular methionine is not required for DNA methylation in the cultured blastocyst. Nevertheless, gene expression changes caused by alteration of DNA methylation (i.e., absence of the methylase genes) can result in embryo death or developmental defects in preimplantation embryos [36].

The hypothesis of the present study was that dietary supplementation with RPM and RPC, or both, increases DNA methylation in preimplantation embryos in dairy cows and is beneficial to embryonic development. The objective of this study was to determine the effects of methionine and choline on DNA methylation and lipid accumulation in preimplantation embryos of Holstein cows.

2. Materials and methods

The Institutional Animal Care and Use Committee from the University of Illinois (Urbana-Champaign, IL, USA) approved all procedures performed in this experiment.

2.1. Experimental design and sample collection

A total of 36 pregnant Holstein cows entering their second or greater lactation were used (parity 2.9 ± 0.2). During the prepartum period, cows were housed in free stalls with individual Calan feed gates (American Calan Inc., Northwood, NH, USA). Approximately, 2 days before expected parturition, cows were moved to individual maternity pens in the same barn until parturition. After parturition, cows were housed in tie stalls with mangers designed for measurement of feed intake. Cows were milked three times daily. Values for milk yield were: week 1 to 4 = 41.1 kg/d (range: 38.1–42.3 kg/d) and week 5 to 10 = 46.4 (44.4–48.3 kg/d). During the experimental period, cows were fed for ad libitum intake. Diets (prepartum and postpartum) were formulated to meet or exceed cows requirements according to NRC [15] and were delivered once daily as a total mixed ration (TMR).

All cows received the same basal close-up diet (1.54 Mcal/kg of dry matter [DM], 18.0% crude protein [CP]) from 21 days before the expected calving, the same basal fresh cow diet from calving (1.71 Mcal/kg of DM, 17.6% CP) through 30 days in milk (DIM), and the same basal high cow diet (1.69 Mcal/kg of DM, 18.3% CP) from 31 to 72 DIM. At 21 days before calving, cows were randomly assigned to one of four treatments (TRT), given as a top-dress on a TMR: supplementation with methionine (MET; n = 9; RPM at a rate of 0.08% [w:w] of the DM, Smartamine M [Adisseo, Alpharetta, GA, USA]), choline (CHO; n = 8; received 60 g/d of RPC, Reashure [Balchem Corporation, New Hampton, NY, USA]), both feed supplements (MIX; n = 11; RPM at a rate of 0.08% [w:w] of the DM [Smartamine M] and 60 g/d of RPC [Reashure]), or no supplementation (CON; n = 8). Cows were randomly reassigned to receive one of two new feed regimens from 30 ± 1 to 72 ± 1 DIM. The two new groups (GRP) were control (CNT; n = 16, fed a basal diet) and methionine (SMT; n = 20, fed the basal diet plus RPM at a rate of 0.08%